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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências e Tecnologias da Saúde - realizada sob a orientação científica do Professor Doutor José Artur Paiva, Professor Associado Convidado do Departamento de Medicina da Faculdade de Medicina da Universidade do Porto, e Professor Doutor José Luís de Almeida, Professor Afiliado do Departamento de Farmacologia e Terapêutica da Faculdade de Medicina da Universidade do Porto.

Para o João

Para a Ana

Para a Marta

o júri

presidente	Professor Doutor **José Rodrigues Ferreira da Rocha** Professor Catedrático, Universidade de Aveiro
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A realização de uma Tese de Doutoramento implica, além do natural esforço e empenho do seu autor, a colaboração de várias pessoas, quer através da participação directa ou indireta nos diferentes passos da mesma, quer pelo seu incentivo ou tão só pela sua paciência, durante um caminho nem sempre fácil e em que as dúvidas e os períodos de menor ânimo são uma constante. Levar a cabo um empreendimento desta natureza torna-se ainda mais difícil quando cumulativamente há que atender outras responsabilidades a nível profissional exigindo tanta dedicação e tempo como a atividade médica em geral, no meu caso particular com acrescidas funções de coordenação clínica e cirúrgica de uma Unidade de Queimados. O presente trabalho pode ser visto neste contexto como resultado do empenho de uma equipa não institucionalizada, mas real e ativa, sendo da mais elementar justiça manifestar aqui a minha profunda gratidão a todos os que generosamente me apoiaram neste projeto.

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palavras-chave

queimaduras, sepsis, biomarcadores, procalcitonina, terapêutica antimicrobiana

resumo

A sepsis, desencadeando disfunção multiorgânica, é a principal causa de morte em doentes queimados, pelo que a instituição de terapêutica antimicrobiana precoce e apropriada é fundamental. Todavia, a dificuldade em distinguir entre um quadro de sepsis verdadeira e uma resposta inflamatória fisiológica à queimadura faz com que muitas vezes o tratamento inicial destes doentes não seja o mais adequado, causando um atraso no início da terapêutica, aumentando a mortalidade ou levando à prescrição antimicrobiana supérflua, com consequente aumento da incidência de efeitos adversos e resistências microbianas. Diversos biomarcadores clínicos e/ou laboratoriais têm sido utilizados para ajudar no diagnóstico de sepsis em outros contextos, particularmente a nível dos Serviços de Urgência e de Cuidados Intensivos. Entre os biomarcadores habitualmente disponíveis, a procalcitonina (PCT) é reconhecida como sendo a mais fiável para essa função. O objetivo principal desta tese consistiu na avaliação do potencial papel da PCT nos protocolos de otimização da terapêutica antimicrobiana em doentes queimados.

Com base numa amostra de doentes de uma Unidade de Queimados de um hospital terciário, e utilizando uma definição de sepsis específica para este tipo de doentes, demonstrou-se que a PCT foi superior aos biomarcadores tradicionais (contagem leucocitária, contagem plaquetária, protrombinemia, D-dímeros, proteína C-reativa, lactato sérico e temperatura) no diagnóstico precoce de sepsis. Foi proposto um limiar de 0,5 ng/mL como determinante da necessidade de avaliação diária da PCT, sendo recomendada terapia antimicrobiana empírica acima de 1,0-1,5 ng/mL. A PCT demonstrou uma correlação forte e estatisticamente significativa com a mortalidade. A persistência de valores de PCT elevados durante a terapêutica antimicrobiana mostrou correlação com a ineficácia desta, opostamente ao sucedido quando esses valores declinaram de forma consistente.

A cinética da PCT mostrou-se de grande valia para o diagnóstico diferencial entre a sepsis e a resposta inflamatória precoce associada a queimaduras, bem como para o diagnóstico de sepsis pós-operatória. Os níveis de PCT foram significativamente mais elevados em doentes com sepsis por bactérias Gram-negativo em comparação com os controlos e com os doentes com um quadro de sepsis por bactérias Gram-positivo. A análise de subgrupos demonstrou ainda que os valores mais elevados ocorreram em doentes com sepsis causada por bactérias Gram-negativas não fermentativas, por *Klebsiella pneumoniae* e, em menor escala, por outras Enterobacteriáceas. Valores de PCT inferiores a 0,5 ng/mL praticamente excluíram as infecções por bactérias Gram-negativas.

Enquanto não estiverem facilmente disponíveis métodos de identificação microbiológica mais rápidos, mais confiáveis e mais baratos, doseamentos seriados da PCT, capacitando as decisões de prescrição, deverão ser incluídos nos protocolos de administração antimicrobiana em Unidades de Queimados, aumentando a eficácia terapêutica e diminuindo os efeitos adversos, as resistências microbianas e os custos.

keywords

burns, sepsis, biomarkers, procalcitonin, antimicrobial stewardship

abstract

Sepsis, inducing multiorganic dysfunction, is the main cause of death in burn patients. A prompt and appropriate selection of antimicrobial therapy is crucial for their outcome. The difficulty in distinguishing true sepsis from physiological inflammatory response associated to burn injury, strongly contributes to an inadequate management of these patients, potentially leading to delayed antimicrobial therapy, increased mortality, or to superfluous antimicrobial prescription, raising the incidence of adverse events and microbial resistance. Several clinical and/or laboratorial biomarkers have been used to help clinicians to distinguish sepsis from systemic inflammatory response, namely at the Emergency and Intensive Care Departments. Among the available biomarkers, procalcitonin (PCT) is recognized as the most reliable for this purpose. The main objective of this thesis was to investigate the potential role of PCT as part of antimicrobial stewardship programs in burn patients.

Taking a sample of patients from a Burn Unit of a tertiary care hospital and using specific burn sepsis definition, the results showed that PCT compared with traditional biomarkers (leucocyte and platelet countings, prothrombinemia, D-dimers, C-reactive protein, serum lactate and temperature) was the best biomarker for an early diagnosis of sepsis. An alert cut-off of 0.5 ng/mL was proposed as reason for daily PCT assessment, with empirical antimicrobial therapy recommended for values above 1.0-1.5 ng/mL. PCT demonstrated a close and statistically significant correlation with the mortality. Sustained increased values during antimicrobial therapy showed a correlation with therapeutic failure, as opposed to what happened when PCT levels consistently fell.

PCT kinetics proved to be of great value for the differential diagnosis between sepsis and early inflammatory response associated with burn injury as well as for the diagnosis of postoperative sepsis in these patients. PCT levels were found to be significantly higher in patients with Gram-negative sepsis comparing to patients with Gram-positive sepsis and controls. Subgroup analysis showed that the most elevated values occurred in patients with sepsis caused by non-fermentative Gram-negative bacteria, by *Klebsiella pneumoniae* and, in a lesser extent, by other Enterobacteriaceae. PCT values under 0.5-ng/mL almost excluded infections due to Gram-negative bacteria.

While faster, more reliable and cheaper methods of microbiological identification are not developed and widely available, repeated PCT measurements, coupled with careful anamnesis and clinical examination, empowering prescription decisions, should be included in antimicrobial stewardship programs in Burn Units in order to increase antimicrobials effectiveness, to reduce mortality, to avoid adverse events and the development of microbial resistance, and to minimize the financial burden.

Contents

Chapter 1 Introduction

1. Background

1.1. Sepsis is the main cause of death in burn patients

Severe burn injuries, affecting all body systems and their regulatory pathways, may be considered as a paradigm of polytraumatism. Tissue injury, coupled with the release of multiple local and systemic mediators of inflammation, leads to an increase in vascular permeability, resulting in huge hydroelectrolytic and cardiovascular changes [1]. These changes rapidly evolve to a state of hypovolemic shock, with loss of water, proteins and electrolytes, which is usually fatal if not adequately treated. In the past, shock used to be the first cause of death in these patients. However, the great advances in intensive care in the last decades have improved the outcome and the initial acute phase of hypovolemia is overcome with success in most cases [2]. Nowadays, sepsis, the systemic infection state evolving to multiorganic failure, became the major cause of death in burn patients, generally occurring in the late post-traumatic period [3].

In the literature, it was referred that burn patients have up to a 3-fold higher prevalence of sepsis than other trauma victims [4]. Comparing to other critical patients, severe burn victims have a higher susceptibility to complications leading to sepsis, due to intrinsic and extrinsic factors [5,6]. The intrinsic factors include the loss of skin barrier, humoral and cellular immunodepression, the presence of necrotic tissue, bacterial translocation and the diminution of airway clearance, namely when there is an associated inhalation injury. The extrinsic factors comprise the use of invasive devices (intravascular catheters, endotracheal tubes, indwelling bladder catheters, etc.), the prolonged immobilization and the exposition to nosocomial flora.

1.2. The diagnosis of sepsis is complex in the presence of burns

Sepsis is a multifactorial syndrome in which microbiological agents, individual immune response and comorbidities contribute to its development. Sepsis identification is not always straightforward, and the progressive changes verified in its diagnostic criteria put in evidence

the difficulties found to confirm the diagnosis. In the last international consensus conference, held by the Society of Critical Care Medicine (SCCM) and by the European Society for Intensive Care Medicine (ESICM) in 2016, sepsis was defined as life-threatening organ dysfunction caused by a deregulated host response to infection (Sepsis 3) [7]. Host dysfunction is suspected by the presence of an acute change in the quick Sequential Organ Failure Assessment score equal or above 2 points (Table 1).

Table 1. Criteria for the suspicion of sepsis

qSOFA (quick Sequential Organ Falency Assessment) $\geq$ 2
Respiratory rate $\geq$ 25/min
Altered mentation (Glasgow Coma Scale < 13)
Systolic blood pressure $\leq$ 100 mmHg
+
Documented Infection
Positive blood culture or
Deep tissue invasion (biopsy with > 10^5 cfu/g) or
Imagiologically documented infection (X-ray, CT scan, MR scan) or
Clinical response to antimicrobials

cfu – colony forming unit; CT – computed tomography; MR – magnetic resonance

In large burns, the clinical signs and laboratorial findings usually taken to diagnose infection are somewhat confusing and even more difficult to interpret due to the magnitude of the systemic inflammatory response triggered by the burn lesion, which mimics a true septic episode. For instance, hyperthermia is frequent in burn patients due to a deregulation of the thermoregulatory centre induced by the burns, and leucocytosis may not be linked to anti-infectious response, particularly in post-operatory situations. Moreover, Sepsis 3 definition has

not yet been validated for burn sepsis. Currently, the most used criteria for the diagnosis of burn sepsis are still those proposed in 2007 by the American Burn Association, specifying cut-offs for clinical signs and requiring documented microbiological identification [8] (Table 2).

Table 2. American Burn Association definition for sepsis in adult burn patients

Clinical Criteria (≥ 3)

Axillary temperature > 39 ºC or < 36.5 ºC

Heart rate > 110/minute

Respiratory rate > 25/min (> 12 L/min if ventilated)

Thrombocytopenia < 100,000/μL

Hyperglycaemia (if no pre-existing diabetes mellitus):

- Plasma glucose > 200 mL in non insulin-treated patients
- Insulin resistance in insulin-treated patients (>7 UI insulin/h)

Inability to enteral feeding > 24 h

- Abdominal distension
- Enteral feeding intolerance
- Uncontrollable diarrhoea (> 2,500 mL/day)

+

Documented Infection

Positive blood culture or

Deep tissue invasion (biopsy with > 10^5 cfu/g) or

Imagiologically documented infection (X-ray, CT scan, MR scan) or

Clinical response to antimicrobials

UI – international unit; cfu – colony forming unit; CT – computed tomography; MR – magnetic resonance

A major problem is that for most burn treatment facilities the actual conditions for definitive identification of microbiological agents in patient samples are time-requiring, taking 2 to 4 days to deliver results [9].

1.3. Adequate and prompt administration of antimicrobial therapy reduces mortality

As stated by Kumar [10] and confirmed by many other studies, the prompt administration of an adequate antimicrobial therapy, i.e. the right dose of the most effective drug against the causative microorganism(s) in the most appropriate pharmacokinetic and pharmacodynamic conditions, is the most important single factor for the survival of the septic patient, and any delay in antimicrobial therapy is associated with increased mortality. This is particularly true in burn patients who are immunodepressed and have a deficient general state due to hypermetabolism and hypercatabolism and constant loss of proteins, hydration, electrolytes and warm from the denuded areas until their new full cutaneous covering is assured [11,12]. Burn physicians are, therefore, urged to start antimicrobial therapy at the first evidence of infection, a decision that requires a thorough clinical assessment and remarkable clinical expertise.

1.4. Unnecessary antimicrobial therapy promotes microbiological resistance

In the presence of an adequate antimicrobial therapy, microbial countings will fall, making it possible for the body systems to control and resolve the infectious episode. This is, however, a dynamic process: microbes struggle to survive and even with the most effective drugs, there are some microorganisms that will be able to resist to the antimicrobials and spread the mechanism of resistance to other bacteria. To reduce the possibility of emergence and selection of microbial resistance, besides the prompt beginning of the antimicrobial therapy at the first evidence of infection, it is crucial to focus the spectrum of the drug on the pathogen

and to limit the duration of the treatment to the strictly necessary, reducing selective pressure [13]. This strategy has additional advantages, e.g. by allowing a reduction of the potential adverse events, of the costs and, in most cases, also of the length of hospitalization, provided that meanwhile surgical covering of the burns is achieved.

1.5. The utilization of biomarkers can decisively help antibiotic stewardship

The impact of an adequate antimicrobial therapy on survival of sepsis patients makes mandatory the establishment of an antimicrobial stewardship program to optimize drug prescription and reduce microbiological resistance [14,15]. The best strategy couples quick identification of infection with timely collection of specimens for microbiological exams and institution of a broad microbiological spectrum antimicrobial therapy. With the attainment of microbiological identification and susceptibility tests antimicrobial therapy should be changed to a narrower spectrum drug capable of attaining cure of the infection and reducing deleterious ecological impact (de-escalation) [16,17]. The extension of the treatment will ideally be the shortest able to produce cure and avoid infection recurrence, which is not easy to define and largely varies for different microorganisms and different patients. Again, even taking into account the importance of microbiological confirmation of infection, the delay in obtaining the results may give rise to unnecessary and potentially deleterious prolongation of therapy. Until the full development and wide availability of new techniques allowing quicker microbiological identification is reached, the use of biochemical and/or biophysical markers of sepsis [18], obviously linked with sound clinical observation, may help the decision on starting and ending the antimicrobial therapy [19], avoiding unnecessary medication, reducing adverse side effects of drug therapy and the selection of resistance, decreasing costs [20], and, in many cases, diminishing the length of hospitalization of burn patients.

1.6. Procalcitonin is one of the best biomarkers for antimicrobial stewardship in septic patients

From a plethora of sepsis biomarkers described in the literature [21], procalcitonin (PCT) is certainly one of the most studied in the last decades and allegedly has the best discriminative power among the ones generally available at hospital facilities [22,23,24,25]. Regulated by CALC-1 gene, PCT is a 116-amino acid peptide precursor of calcitonin, mainly secreted by thyroid C cells, and in healthy individuals it is barely detected in blood (< 0.10 ng/mL). In the presence of systemic infection, CALC-1 is also expressed in nonthyroidal cells all over the body (liver, kidney, adipocytes, etc.) and consequently the levels of PCT suffer a sudden and dramatic increase, due to a production in large amounts of PCT that is not cleaved to form calcitonin, as occurs in thyroid C-cells, being abruptly released to the bloodstream, till 1000 times its usual concentration [26]. Serum PCT increase is noticeable just 2-4 hours after sepsis onset, peaks at 24-48 hours and its values follow the course of the infection, quickly subsiding with the control of the septic process, in average diminishing around 50% every 1-1.5 days [27]. PCT has been tested against other available biomarkers, like C-reactive protein (CRP) and interleukin IL-6 and it seems to be more accurate and more suitable to diagnose and monitor several infectious processes and its treatment [28]. Recent meta-analysis have confirmed a good correlation between abnormal PCT levels and the presence of bloodstream infections and that elevated PCT levels and also that PCT non-clearance is related with an increased risk of sepsis and a higher mortality rate [29,30]. On the other hand, some authors have reported that the different composition of the membrane cells of Gram-negative, Gram-positive and fungi elicit the activation of different types of cytokines leading to diverse degrees of PCT production [31,32]. These different patterns might give some hints about the causative microorganisms, while microbiological identification is not available [33].

Considering its fair accuracy for sepsis diagnosis and the correlation of its kinetics with sepsis evolution [34], PCT use has been recommended in diverse clinical contexts, such as the exclusion of bacterial involvement in lower respiratory infections [35], the diagnosis, stratification, prognostic assessment [36,37] and antimicrobial stewardship of septic patients [38,39], the diagnosis of postoperative infections [40,41,42]; the differential diagnosis of sepsis from diverse microbial origins [43,44], etc.

The utility of PCT for management of burn patients has, however, been questioned due to its mild elevation in localized infections, and to a substantial amount of false positives originated by the burn-associated or surgery-associated systemic inflammatory response [45]. These would limit its interest for antimicrobial stewardship in burn sepsis patients, which has not been adequately studied. Indeed, most of the published literature on the use of PCT rarely includes significant number of burn patients, usually analyzed together with other critical care patients, with much diverse clinical features and also employing diverse sepsis definitions [46,47].

In 2007, Tang et al. [48], in a meta-analysis pooling 2,097 patients, in which the presence of burns was an exclusion criterion, concluded that their study did not support a widespread use of PCT for sepsis diagnosis in critical care settings and stated that this could not be generalized to burn patients. In the PASS study [49], performed in 2011, in nine Danish intensive care units (ICUs) and including 1,200 patients, most patients came primarily from medical wards and there is no reference to the inclusion of burn patients. In their controversial work, the authors concluded that the use of PCT for antibiotherapy escalation did lead to organ-related harm and prolonged length-of-stay at the ICU. In spite of it, the study confirmed that PCT levels may be taken as a good predictor of mortality. In a study published in 2014, using a sample of 34 burn patients with only 16 patients with documented infection, Seoane et al. [50] concluded that PCT was not a precise indicator of sepsis. However, besides the reduced sample size, they did

not perform an analysis of PCT kinetics, merely using samples from the day of sepsis suspicion, the day before and the day after, and no information was provided about potential ongoing antimicrobial therapy.

On the other hand, in 2011, in a meta-analysis by Mann et al. [51] including burn patients exclusively, the authors stated that PCT assay can be used to help sepsis diagnosis and to reduce antibiotic exposure. Lavrentieva et al. [52], in a prospective study published in 2012, with 145 patients and daily PCT measurement, found that PCT kinetics has great diagnostic and prognostic value, and is also a valuable tool in monitoring the efficacy of antimicrobial therapy. In 2015, Ren et al. [53] published a meta-analysis, including 566 burn patients from nine trials, concluding that, despite some heterogeneity among these studies, PCT was a useful biomarker for sepsis diagnosis.

2. Thesis Aims and Structure

Sepsis, inducing multiorganic dysfunction, is the main cause of death in burn patients. A prompt and appropriate selection of antimicrobial therapy is crucial. The difficulty in distinguishing true sepsis from physiological inflammatory response to burn injury, strongly contributes to an inadequate management of these patients. The main objective of this thesis was to investigate the utility of PCT as a biomarker for antimicrobial stewardship in burn patients**.**

To achieve its goal, the thesis was organised in seven chapters. The first one is the introduction of the subject, which is followed by five chapters corresponding to five articles published in peer-reviewed, indexed, journals: one meta-analysis assessing PCT use in Burn Units in the world and four clinical studies analysing the performance of PCT in different perspectives, using samples of burn patients from Coimbra Burns Unit (CBU), from the Plastic Surgery and Burns Department of Coimbra University Hospital Centre (Centro Hospitalar e Univesitário de

Coimbra – CHUC), in Portugal. The seventh and last chapter is dedicated to global discussion and presentation of the conclusions.

In Chapter 1, the background picture of burn sepsis was drawn, exposing the current concepts about its pathophysiology, the difficulties existing for its diagnosis and prognosis as well as the complexities associated to its management and the paper that biomarkers, namely PCT, may have in this context. PCT potential for the monitoring of antimicrobial therapy efficacy was also referred.

First of all, and in order to appraise the best available evidence, in Chapter 2 the author performed a systematic review of medical literature about the utilization of PCT measurement in the managing of sepsis in burn patients. Taking in account the scarce number of studies that were found, with relatively small and heterogenous populations, a meta-analysis was also done fulfilling all the criteria of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist [54]. Two meta-analysis methods were used to calculate the pooled effect estimates: the inverse variance assuming a fixed-effects model, and the DerSimonian-Laird method assuming a random-effects model. Homogeneity among studies was evaluated using the Cochran's Q statistic and the I2 statistic. The publication bias associated with the AUC on diagnostic sepsis effect was analysed by the funnel plot and the Egger test.

Trying to overcome the uncertainty derived from the heterogeneity of populations on the existing studies, and its small sample sizes, it was decided to perform a set of clinical studies based in a large sample of patients from Coimbra Burn Unit, using the ABA criteria for the inclusion/exclusion of patients. A total of four clinical studies, covering different but complementary aspects important to assess the role of PCT as biomarker for antimicrobial stewardship in burn patients were accomplished, evaluating PCT potential for assessing the diagnosis, prognosis, antimicrobial therapy monitoring, and Gram type of causative microorganisms in burn sepsis patients. The results were published in indexed and peer-reviewed scientific journals, and reprints from the published papers are presented in this

thesis, corresponding to Chapters 3 to 6.

In Chapter 3, PCT performance for the early diagnosis of burn sepsis is appraised, using ROC (Receiver Operative Characteristics) curves for comparison with other currently employed infection biomarkers (leucocyte and platelet countings, prothrombinemia, D-dimers, C-reactive protein, serum lactate and temperature), with a view to validating its use for the sepsis diagnosis in burn patients. Under a nonparametric approach, the quantitative variables were analysed with Mann–Whitney U test and qualitative variables were analysed with Pearson chi-square test. To measure the effect-size the probability of superiority (PS) was used. A cut-off for sepsis diagnosis in this sample of CBU patients was proposed using Youden Index.

The feasibility of PCT use to predict the outcome and to monitor the efficacy of antimicrobial therapy in a sample of severe burn adult patients is sized up in Chapter 4. Again, quantitative variables were analysed with Mann–Whitney U test and qualitative variables were analysed with Pearson chi-square test, while time variations of PCT levels were tested using Friedman's test and Kendall's W.

Chapter 5 presents an observational retrospective study done to investigate whether or not the alterations of inflammatory features directly resulting from tissue trauma caused by burns in the acute phase, and also due to surgical interventions, would interfere with the ability of PCT to distinguish between this pure inflammatory response and septic conditions. Comparisons between sepsis and no sepsis groups were performed using the Mann-Whitney test for quantitative variables and the Fisher's exact test for qualitative variables, while time comparisons used Friedman's test.

In Chapter 6, the accuracy of PCT to distinguish sepsis caused by Gram-negative and Gram-positive bacteria in burn patients is assessed. Kruskall–Wallis and Mann–Whitney tests were used to compare quantitative variables. Qualitative variables were compared with the Pearson chi-square test. For pairwise comparisons, the Bonferroni correction was applied. ROC curves were performed, paying attention to the areas under the curve (AUC) for Gram negative and

Gram-positive sepsis and subgroup analysis according to the most common microorganisms in each group were also done.

Chapter 7 is dedicated to the integrated discussion of the studies described above, finally drawing and presenting the conclusions on the usefulness PCT for antimicrobial stewardship in Burn Units.

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Chapter 2 The use of procalcitonin (PCT) for diagnosis of sepsis in burn patients: a meta-analysis

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RESEARCH ARTICLE

The Use of Procalcitonin (PCT) for Diagnosis of Sepsis in Burn Patients: A Meta-Analysis

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Abstract

The continuous development of resuscitation techniques and intensive care reduced the mortality rate induced by the initial shock in burn patients and, currently, infections (especially sepsis) are the main causes of mortality of these patients. The misuse of antimicrobial agents is strongly related to antimicrobial and adverse patient outcomes, development of microbial resistance and increased healthcare-related costs. To overcome these risks, antimicrobial stewardship is mandatory and biomarkers are useful to avoid unnecessary medical prescription, to monitor antimicrobial therapy and to support the decision of its stop. Among a large array of laboratory tests, procalcitonin (PCT) emerged as the leading biomarker to accurately and time-effectively indicate the presence of systemic infection. In the presence of systemic infection, PCT blood levels undergo a sudden and dramatic increase, following the course of the infection, and quickly subside after the control of the septic process. This work is a meta-analysis on PCT performance as a biomarker for sepsis. This meta–analysis showed that overall pooled area under the curve (AUC) is 0.83 (95% CI = 0.76 to 0.90); the estimated cut-off is 1.47 ng/mL. The overall sepsis effect in PCT levels is significant and strong (Cohen's d is 2.1 and 95% CI = 1.1 to 3.2). This meta–analysis showed PCT may be considered as a biomarker with a strong diagnostic ability to discriminate between the septic from the non-septic burn patients. Thus, this work encourages the determination of PCT levels in clinical practice for the management of these patients, in order to timely identify the susceptibility to sepsis and to initiate the antimicrobial therapy, improving the patients' outcomes.

Introduction

Comparing to other critical patients, severe burn victims have a higher susceptibility to develop infectious complications leading to sepsis, which is the major cause of mortality in these patients, and may result from intrinsic and extrinsic factors [1,2]. The former may include loss of skin barrier, humoral and cellular immunodepression, presence of necrotic

tissue, bacterial translocation and diminution of airway clearance when inhalation injuries are associated. The later comprise the use of invasive devices (intravascular catheters, endotracheal tubes, indwelling bladder catheters, etc.), immobilization and exposition to nosocomial flora [2,3].

Clinical signs and laboratorial findings commonly used to diagnose the presence of infection are not specific and are difficult to interpret due to the magnitude of the systemic inflammatory response unfettered by large burns, which mimics a septic episode. The consensus international definition of sepsis, formulated by the American College of Chest Physicians and by the Society of Critical Care Medicine (ACCP/SCCM) [4,5], was subjected to a revision for burn patients by the American Burn Association [6] (see Annex 1) [S1 File]. This revision implied the modification of some cut-offs and the concomitant documentation of microbiological identification. Nevertheless, currently, the definite identification of microbiological agents still takes two to four days [7]. As stated by Kumar [8] and confirmed by many other studies, the prompt administration of an adequate antimicrobial therapy is the most important isolated factor for the survival of the septic patient and any hourly delay is associated with an increase in mortality.

Burn surgeons are therefore urged to start antimicrobial therapy at the first evidence of infection, but it requires a strong clinical expertise, attending to the lack of a time-effective microbiological confirmation. An adequate therapy reduces the microbial counting, which enables the body systems to control and stop the infectious episode. This is, however, a dynamic process: there are resistant microorganisms that may survive, even when treated with the most effective bactericidal agents. Some microorganisms develop mutations capable of overlapping the antibiotic action, giving rise to microbial resistance (i.e. making the drug ineffective), and thus they may spread to other cells and tissues, being responsible for a systemic infection. To reduce the possibilities of development of microbial resistance is crucial to avoid unnecessary administration of antimicrobial therapy. On the other hand, the prompt beginning of the therapy, with the right dose of an effective drug at the first evidence of infection is equally important, so are the selection of the right drug targeting the microbiological agent and to limit the duration of treatment to the strictly necessary, preventing antibiotic resistance and selective pressure on the microorganisms [9]. This strategy has additional advantages, including the reduction of medication side effects, healthcare-related costs and, in most cases, the length of hospitalization (providing that the surgical treatment of the burns is achieved).

The use of biomarkers has been recommended to help clinicians to timely decide when to start antimicrobial therapy, to monitor its evolution and to advise its early suspension. From the currently available biomarkers, procalcitonin (PCT) has shown the greatest accuracy to indicate the presence of systemic infections within an acceptable timing, in a great range of clinical scenarios [10–12].

This work is an extended and updated version of the paper published by Ren *et al.* [13] [S2 File], including the overall estimation and discussion of several other effect sizes in PCT levels and incorporating four other studies. Its aim is to summarize literature data (through meta-analysis) about the use of PCT for the early detection of sepsis in burn patients, and to discuss the proposed PCT cut-offs for the diagnosis of sepsis.

Material and Methods

Data source

PubMed, Scopus and Web of Science databases were used. The combined search term used for this search was: [procalcitonin OR PCT) AND (sepsis OR septic) AND burn]. The search was performed up to 1st December 2015.

Data extraction, evaluation and synthesis

Only articles written in English focusing on burn patients and on the evaluation of PCT role on the diagnosis and monitoring of septic episodes were considered. Titles and abstracts of records retrieved by the search were screened to determine their relevance. Relevant studies were reviewed in full text, in order to determine their relevance for the meta-analysis. After reading titles and eliminating duplicates (LC and VA), 96 abstracts were independently assessed by three authors (LC, VA and LA), and, from these, 14 references were subjected to detailed analysis and included in the sample, by consensus or majority decision.

Inclusion and exclusion criteria

A study was considered eligible for inclusion in the meta-analysis if it provided area under the curve (AUC) on serum PCT for diagnosis of sepsis or the serum PCT levels by sepsis and non-sepsis groups in burn patients.

Statistical analysis

Two techniques were used to calculate the pooled effect estimates: the inverse variance assuming a fixed-effects model, and the DerSimonian-Laird method assuming a random-effects model.

Homogeneity among studies was evaluated using the Cochran's Q statistic and the I^2 statistic (the values of 0.25, 0.50, and 0.75 indicating low, moderate, and high degrees of heterogeneity). Publication bias was evaluated using the funnel plot and the Egger regression asymmetry test.

To investigate potentially different effects according to the study, subgroup analyses were performed. Sensitivity analysis to show the impact of each study or subgroup studies on the results was also held.

Meta-DiSc 1.4 (XI Cochrane Colloquium, Barcelona, Spain) was used to calculate the summary receiver operating characteristics (SROC) and the pooled AUC [14]. MetaXL 2.0 (EpiGear International Pty Ltd, Wilston, Queensland, Australia) was used to calculate the pooled Cohen's d effect sizes (difference of PCT levels between sepsis and non-sepsis groups, the pooled AUC and pooled mean effects [15].

The weight average of all PCT cut-off for sepsis diagnosis proposed in the studies under analysis was also measured.

Results

The removal of duplicates from the 160 articles that were initially identified through search in PubMed, Scopus and Web of Science resulted in 96 individual articles (Fig 1). The great majority did not fulfill the eligibility criteria. After exclusion of ineligible papers, 14 articles, comprising a temporal range from 1997 to 2015, were found to meet the inclusion criteria and were selected for review.

Plasma PCT concentrations had been measured using different methods, such as PCT-Q immunochromatography (Brahms Diagnostica, Berlin, Germany) [15,16], PCT-Lumi immunoluminometric (Brahms Diagnostica, Berlin, Germany) [16–21], electrochemical luminescence immunoassay (ECLIA) (Brahms Diagnostica, Berlin, Germany) [22–24], and immunoassay sandwich and final fluorescence technique (VIDAS, bioMérieux, Marcy L'Etoile, France) [25].

Two studies were pediatric [20,26], one mixed [18] and the remaining studies included only adult patients [15–19,21,23–25,27–29].

Studies also differ in the PCT cut-off defined for sepsis suspicion. Reported cut-off values include 0.5 ng/mL [23], 0.534 ng/mL [16], 0.69 ng/mL [22], 1.5 ng/mL [19,27], 1.7 ng/ mL

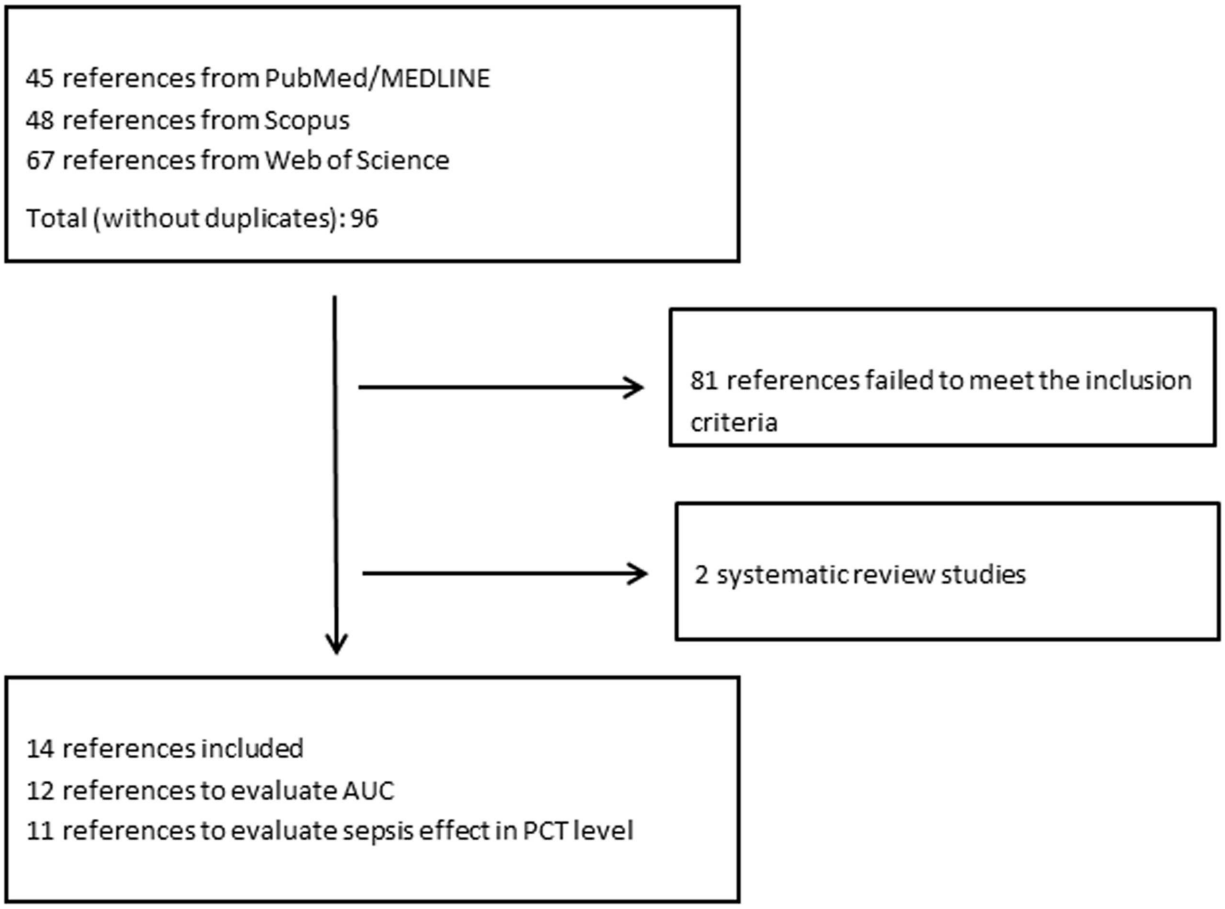

Fig 1. Flow chart for the selection process of studies for evaluation of procalcitonin (PCT) in sepsis diagnosis.

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[23], increment of 1.5 ng/mL in two consecutive days [18], 2 ng/mL [25,29]; 3 ng/mL [17], and 5 ng/mL [20]. Using the different cut-offs for sepsis diagnosis proposed in each study (Table 1), the weight average of all PCT cut-offs for sepsis was computed and the resulting cut-off was 1.59 ng/mL. Fig 2 shows a bubble plot of cut-off for PCT in sepsis diagnosis for 12 studies organized by year. The two older studies showed the highest cut-offs values; if these two studies are excluded, the estimated value is 1.47 ng/mL.

Data uniformization

Data uniformization is required for the meta-analysis of Cohen's d effect size. In the study of Sachse *et al.* [18], PCT values were reported as the median by different post-burn time intervals (6 distinct intervals) for septic and non-septic groups; the average and the standard deviation of PCT values were deduced assuming the normal behavior of PCT values ($\text{mean} = \frac{\sum \text{median}_i}{6}$; $\text{std} = \sqrt{n}\ \text{std}_{\text{median}}$).

In Neely *et al.* [20] and Lavrentieva *et al.* [27] (both in sepsis and non-sepsis groups PCT standard deviation by each group was obtained using the inter-quartile distance and assuming the normal behavior of PCT values (interquartile range = 1.35σ). In Cakir Madenci *et al.* paper [22], it was calculated using the quantiles 2.5% and 97.5% and the normality assumption ($x_{0.975}$-$x_{0.025} = 3.92\sigma$).

Table 1. Area under the curve (AUC) and the corresponding standard error (SE) for each study evaluating the ablity of procalcitonin (PCT) as a biomarker, and the overall estimate using random effects model.

Study	Cut-off (ng/mL)	Time points	N	ROC AUC	SE	95%CI	Tp	Fp	Fn	Tn
Sachse, 1999 (	N/A	N/A	19	N/A	N/A	N/A	N/A	N/A	N/A	N/A
von Heimburg, 1998	3	27	27	N/A	N/A	N/A	2	0	16	9
Neely, 2004	5	62	20	N/A	N/A	N/A	11	12	15	24
Abdel-Hafez 2007	N/A	N/A	42	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bargues, 2007	0.534	359	25	0.66	0.04	0.59–0.72	39	29	53	237
Lavrentieva, 2007	1.5	934	43	0.98	0.03	0.91–1.04	93	72	21	748
Barati, 2008	0.5	60	60	0.97	0.02	0.93–1.01	30	3	0	27
Bognar, 2010	2	196	28	0.77	0.03	0.70–0.83	73	32	11	78
Lavrentieva, 2012	1.5	139	145	0.97	0.01	0.94–0.99	64	5	9	67
Kim, 2012	2	175	175	0.84	0.03	0.79–0.90	72	15	21	67
Cakir Madenci, 2013	0.759	611	37	0.85	0.02	0.81–0.88	181	79	59	292
Seoane, 2014	1.7	34	34	0.55	0.11	0.33–0.77	4	0	12	18
Paratz, 2014	1.4	345	54	0.62	0.04	0.54–0.70	38	190	10	106
Mokline, 2015	0.69	121	121	0.93	0.03	0.87–0.98	39	12	5	65
Total (AUC random effects)				0.83	0.04	0.76–0.90				
Q				182.0						
p-value				<0.001						
I^2				95%						

N—total number of individuals; ROC AUC—receiver operating characteristic area under the curve; 95%CI—95% confidence interval; Fn—false negative; Fp—false positive; N/A–not available; Tn—true negative; Tp—true positive.

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In Bargues *et al.* [16], log values were transformed and combined in order to obtain the PCT average and standard deviation, and the subgroup values are combined to obtain the pooled standard deviation and the weight average. Lavrentieva *et al.* [19] combined subgroup values to obtain the pooled standard deviation and the weight average of PCT for non-sepsis groups.

As Barati *et al.* [15], Bognar *et al.* [29] and Mokline *et al.* [21] did not present AUC standard error, the estimates for standard error were computed using the Hanley and McNeil procedure [30]. Paratz *et al.* [24] reported the PCT discriminative power as not significant with AUC = 0.38 (95% Confidence Interval (95% CI) 0.29 to 0.46). However, if the classifier was negated on every instance, the true positive (TP) classifications become false negative and the false positive become true negative (TN), and we obtain AUC = 0.62 and, for the cut-off chosen by the authors (1.4 ng/mL), the corresponding sensitivity is 80 and the specificity is 36.

In Lavrentieva *et al.* [27], Kim *et al.* [25], Cakir *et al.* [22], Seoane *et al.* [23] and Paratz *et al.* [24], the standard error is estimated from AUC confidence interval (SE = (UB-LB) / 3.92). Table 1 presents the estimate AUC and the corresponding standard error for each study.

To evaluate PCT as a diagnostic marker for sepsis, there are several studies based on time-points with repeated measures and others with independent ones and, in this context, the results were used as independent.

Meta-analysis

For all effect sizes under analysis, the studies show significant heterogeneity ($p<0.01$, $I^2>50\%$), thus a random-effects model for meta-analysis was used.

AUC plays a central role in evaluating diagnostic ability of tests, in particular of PCT biomarker. Ten studies under analysis present the PCT AUC estimate value and the first four

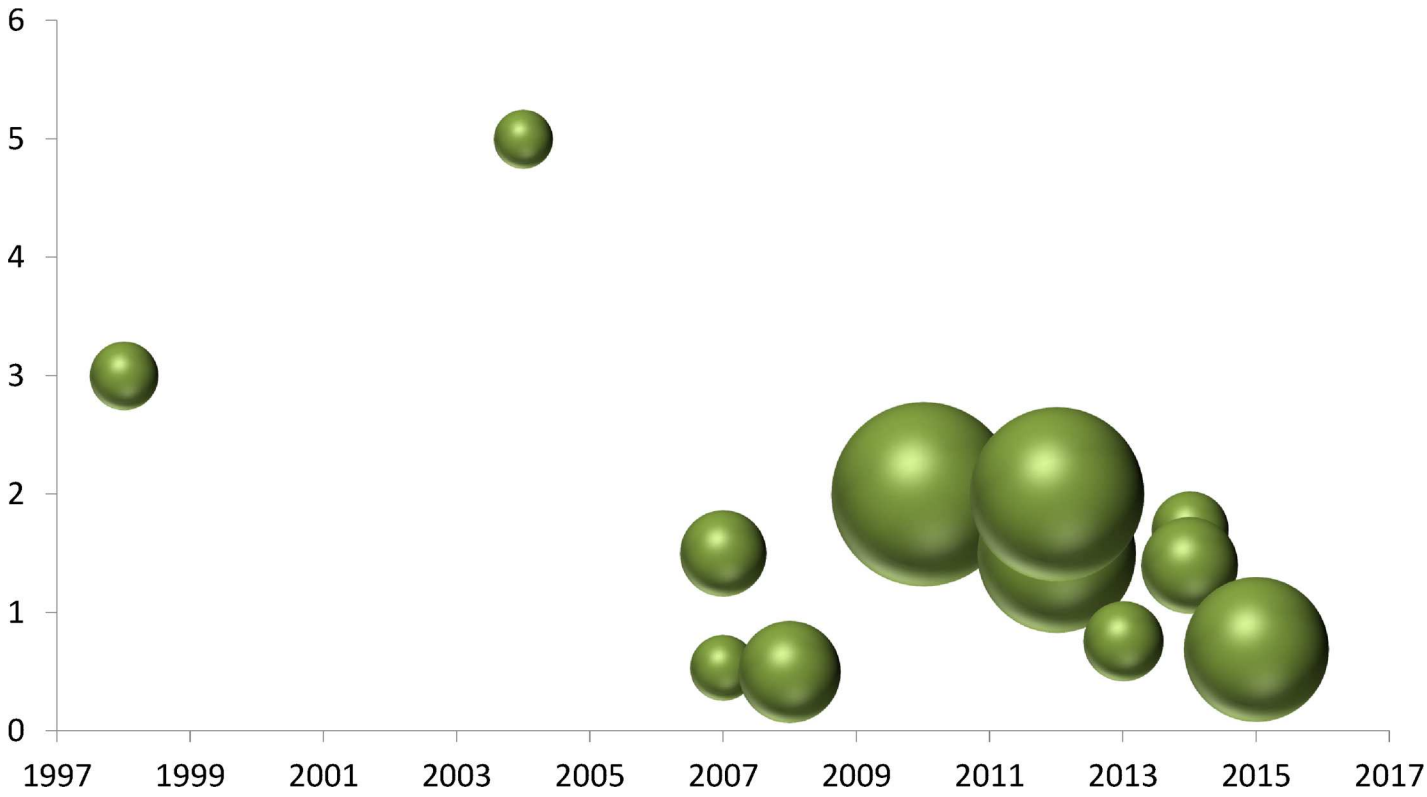

Fig 2. Bubble plot of cut-off for procalcitonin (PCT) in sepsis diagnosis for 12 studies organized by year. Bubble size corresponds to the number of time-points.

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studies reported in Table 1 do not present AUC values. Table 1 presents the overall estimated AUC for PCT for sepsis diagnosis, where the pooled estimate is 0.83 (95% CI = 0.76 to 0.90). PCT diagnosis ability is significant (AUC>0.5) and the effect size is strong.

The publication bias associated with the AUC on diagnostic sepsis effect was analysed by the funnel plot and the Egger test. The result of Egger's test was significant ($p < 0.001$), which is manisfested in funnel plot asymmetry (Fig 3). It is of note that the studies appearing to have higher effect in the publication bias are those which had lower AUC values.

To find out sources of heterogeneity, a subgroup analysis was done, using the random effect model, according to different criteria used for sepsis determination in the works of the sample, namely clinical evaluation, Baltimore Sepsis Scale, American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) definition and the more recent and specific one from the American Burn Association (ABA) (Table 2). In order to reduce subjectivity using standardized concepts, this analysis included just the works explicitly employing the ACCP/CSCCM or the ABA definition. For the former subgroup, the AUC was 0.87 (95% CI = 0.63 to 1.0) and for the later it was 0.87 (95% CI = 0.71 to 0.90).

We also conducted another subgroup analysis, excluding retrospective studies [31,32], achieved an AUC of 0.86 (95% CI = 0.78 to 0.93).

Similarly to the analysis presented by Ren *et al.* [13], the summary receiver operating characteristic (SROC) for PCT in sepsis diagnosis was obtained including all the studies considered (four additional studies to those included in Ren *et al.*). Data reported for SROC estimation by these authors have, however, some differences in comparison to the data used in the present work (Table 1). When the study reported the use of several time-points, the total of time-points

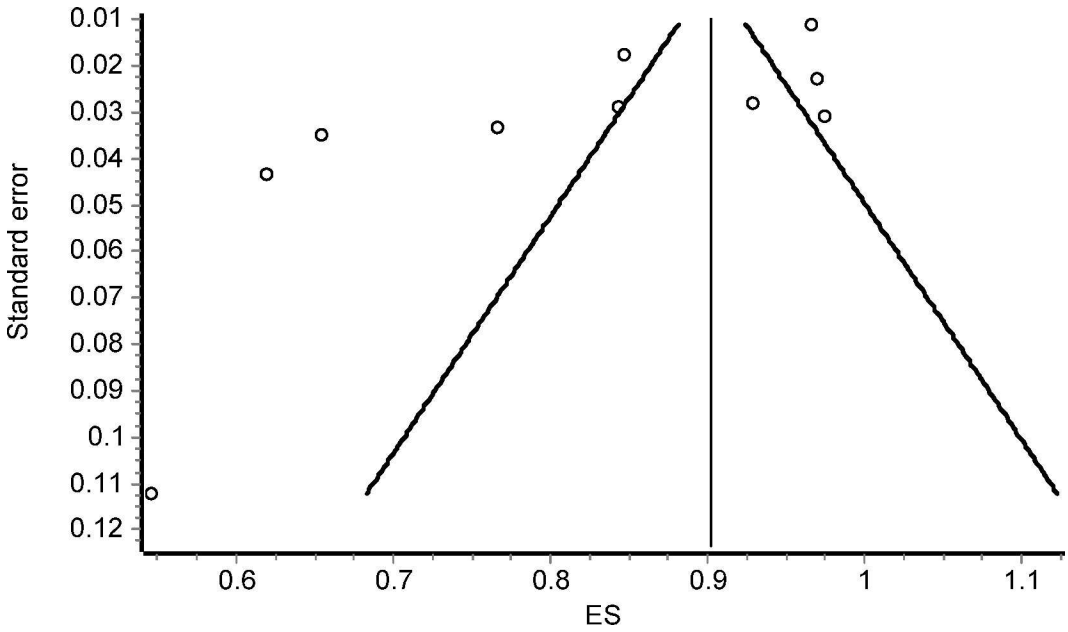

Fig 3. Funnel plot of the AUC on diagnostic sepsis effect

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was used as sample size instead of the total number of individuals [16,19,21,22,24,29]. Moreover, as the revision from Bognar *et al.* [29] was developed including only septic patients, this feature could add some additional bias.

Fig 4 plots the sensitivity vs the false positive rate of all studies (using the values indicated in Table 1), presenting the SROC and achieving an overall AUC of 0.87 (SE = 0.04). The results produced by this method are in accordance with those obtained directly by the DerSimonian-Laird method (Table 1). The pooled sensitivity and specificity are 0.77 (95% CI = 0.72 to 0.80) and 0.65 (95% CI = 0.62 to 0.69), respectively.

PCT mean values for sepsis and non-sepsis groups for eleven individual studies are presented in Table 3. All the studies presenting the values by groups were considered. Due to the

Table 2. Study characterization by sepsis criteria employed, type of design and population age.

Study	Sepsis Criteria	Design Type	Population Age
von Heimburg, 1998	BSS	Prospective	Adult
Sasche, 1999	Clinical	Retrospective	Mixed
Neely, 2004	Clinical	Prospective	Paediatric
Abdel-Hafez, 2007	Clinical	Prospective	Paediatric
Bargues, 2007	ACCP/SCCM	Prospective	Adult
Lavrentieva, 2007	ACCP/SCCM	Prospective	Adult
Barati, 2008	ACCP/SCCM	Prospective	Adult
Bognar, 2010	ABA	Prospective	Adult
Lavrentieva, 2012	ABA	Prospective	Adult
Kim, 2012	Clinical	Prospective	Adult
Cakir Madenci, 2013	ABA	Prospective	Adult
Seoane, 2014	ACCP/SCCM	Retrospective	Adult
Paratz, 2014	ABA	Prospective	Adult
Mokline, 2015	ACCP/SCCM	Prospective	Adult

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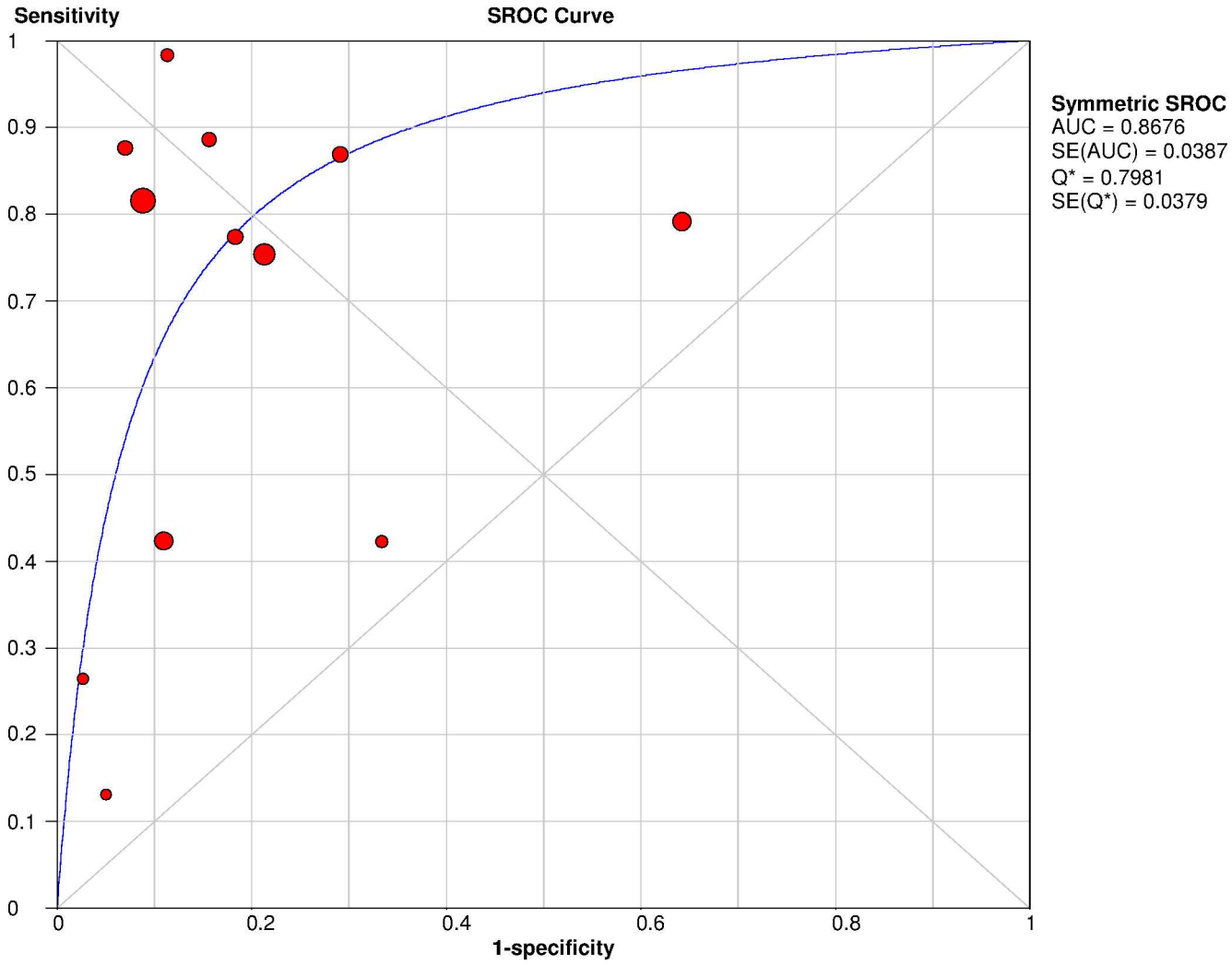

Fig 4. Summary receiver operating characteristic (SROC) curve of procalcitonin (PCT) for the diagnosis of sepsis in burn patients.

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significant heterogeneity, the overall mean estimate was obtained assuming the random effects model: 46.8 ng/mL (95%CI = 2.5 to 91.1) for sepsis group and 0.9 ng/mL (95%CI = 0.1 to 1.6) for non-sepsis group. This analysis is useful to evaluate the strength of the PCT concentration for each group, and a statistically significant mean difference was observed between sepsis and non-sepsis groups.

In the sepsis group, there are three studies with very high PCT concentration (von Heimburg *et al.* [17], Bargues *et al.* [16], Abdel-Hafez *et al.* [26]) (>45 ng/mL); excluding these studies, an overall PCT mean value of 6.4 ng/mL (95%CI = 3.8 to 9.0) was obtained for the sepsis group and of 0.6 ng/mL (95%CI 0.2 to 0.9) for the non-sepsis group. The mean results are robust after the exclusion of these three studies, which perhaps shall be considered as outliers related to different dosing methodology.

Fig 5 shows the mean difference effect sizes (sepsis and non-sepsis group on PCT concentration) for the eleven studies. Two of these studies (Abdel-Hafez *et al.* [26], Bargues *et al.* [16]) reported a much higher difference between groups than the difference observed in the other studies. Due to this clear heterogeneity, a subgroup analysis was performed. Inside both subgroups, the heterogeneity is also significant ($p<0.001$, Cochrane Q test), justifying

Table 3. Procalcitonin (PCT) mean values and the corresponding standard error (SE) for each study, and the overall estimate using random effects model estimated by group (sepsis and non-sepsis group).

	Sepsis group			Non-sepsis group		
Study and year	**Mean**	**SE**	**N**	**Mean**	**SE**	**N**
Sachse, 1999	3.9	11.7	9	0.4	0.4	10
von Heimburg, 1998	49.8	76.9	18	2.3	3.8	9
Neely, 2004	6.7	20.4	36	2.1	3.2	26
Abdel-Hafez, 2007	369.1	11.4	20	47.4	10.7	22
Bargues, 2007	45.5	10.9	92	2.8	1.1	267
Lavrentieva, 2007	11.8	15.8	114	0.6	0.4	820
Barati, 2008	8.5	7.8	30	0.5	1.0	30
Lavrentieva, 2012	7.2	24.1	86	0.7	2.8	53
Cakir Madenci, 2013	2.0	22.0	240	0.3	2.7	371
Seoane, 2014	3.0	5.4	16	0.6	0.3	18
Mokline, 2015	7.3	7.0	44	0.9	0.5	77
Total (random effects)	46.8	22.6		0.9	0.4	
Q	19649			24		
p-value	<0.001			0.004		
I^2	100%			63%		
95%CI	2.49–91.05			0.10–1.61		

N—total number of individuals; 95%CI—95% confidence interval; SE—standard error.

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therefore the use of random effects models. The overall sepsis effect is significant (95%CI = 1.1 to 3.2 with overall estimate of 2.1 ng/mL). Including only the low difference group, the overall effect remains significant, but the effect strength is lower (0.9 ng/mL with 95%CI = 0.2 to 1.5), as expected.

Sensitivity analysis by excluding one study at each turn and pooling results from the remainder further confirmed the robustness of the findings, confirming the significance of the sepsis effect on PCT concentration (Table 4).

The result of Egger's test was not significant (p = 0.194). Thus the publication bias associated to the meta-analysis of difference of PCT levels between sepsis and non-sepsis groups seems to be not relevant. However, the oldest studies included in the meta-analysis of sepsis effect (Fig 5) caused (non-significant) funnel plot asymmetry (Fig 6). Doing again a subgroup analysis based on the used sepsis definition, the resulting values for Cohen's d were 3.69 (95% CI = 0.45 to 6.92) when ACCP/SCCM classification was employed; 0.64 (95% CI = 0.02 to 1.26) according to ABA classification and 3.38 for the rest (95% CI = 0.90 to 5.87).

Discussion

Burns represent a public health problem and are an important cause of mortality and morbidity around the World. According to the World Health Organization (WHO), it is estimated that 265 000 deaths occur every year from fire-related burn injuries. Most of these injuries occur in low- and middle-income countries and almost half of these cases are registered in the South-East Asia Region. Moreover, burns are one of the major causes of disability-adjusted life years (DALYs) lost in these countries. It was estimated in 2004 that nearly 11 million people worldwide were burned severely enough to require medical support. Burns also significantly impact the healthcare-related costs, in particular concerning prolonged hospitalization periods, burn management, and care for disfigurement and emotional trauma [33].

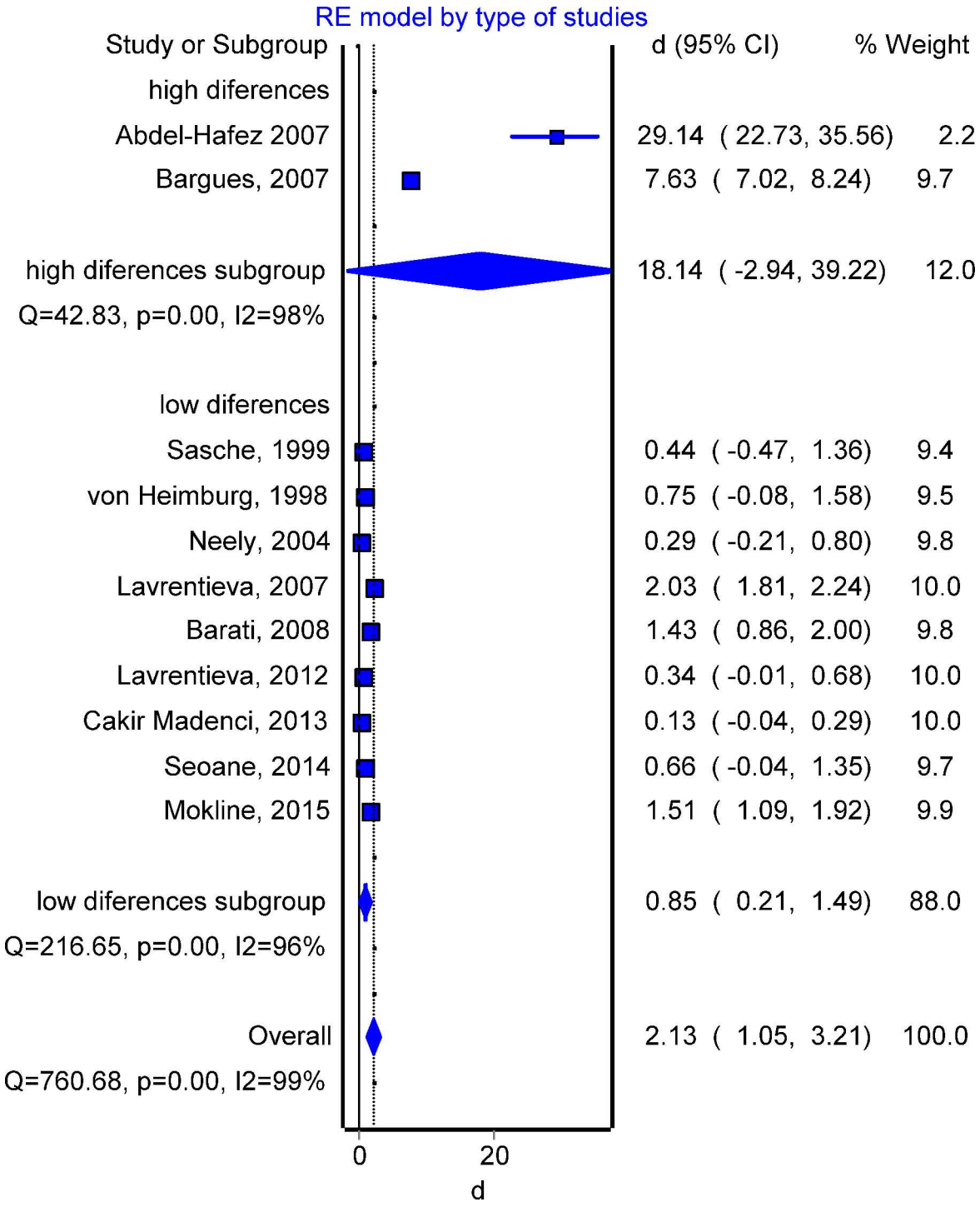

Fig 5. Forest plot for sepsis effect on procalcitonin (PCT) concentration. The estimated overall effect size and confidence interval (Cohen's d, displayed as a diamond) and individual effect sizes (Cohen's d, displayed as a rectangle) are shown.

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Severe burn injuries that affect all body systems and their regulatory pathways may be considered as a paradigm of polytrauma. Tissue injury, coupled with the release of multiple local and systemic mediators of inflammation, leads to an increase in vascular permeability,

Table 4. Sensitivity analysis of overall sepsis effect (Cohen's d) in procalcitonin (PCT) levels in burn patients.

Excluded study	Pooled d	95%CI
Sachse, 1999	2.317	1.170–3.464
von Heimburg, 1998	2.290	1.139–3.442
Neely, 2004	2.365	1.190–3.539
Abdel-Hafez 2007	1.520	0.480–2.559
Bargues, 2007	1.182	0.447–1.917
Lavrentieva, 2007	2.279	0.992–3.566
Barati, 2008	2.237	1.067–3.406
Lavrentieva, 2012	2.404	1.183–3.624
Cakir Madenci, 2013	2.467	1.204–3.730
Seoane, 2014	2.309	1.150–3.468
Mokline, 2015	2.255	1.057–3.453

95%CI–95% confidence interval.

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resulting in marked hydroelectrolytic and cardiovascular alterations [34]. These alterations rapidly evolve to a state of hypovolemic shock, with loss of water, proteins and electrolytes, which is usually fatal if not adequately treated. In the past, shock was indeed the first cause of death in these patients. However, the great advances observed in intensive care have reversed this situation and today this initial acute phase of hypovolemia is overcome with success in the majority of the cases [35]. Nowadays, sepsis has become the major cause of death in burn patients, occurring generally in a late post-traumatic period [35,36].

Considering the patients with suspected infection, septic patients have obviously the worst outcomes [37]. These outcomes may be highly improved, if the appropriate antibiotics are administered early and timely [38]. The use of reliable biomarkers that early identify a septic process may have a great importance to help the physicians to select patients for prompt

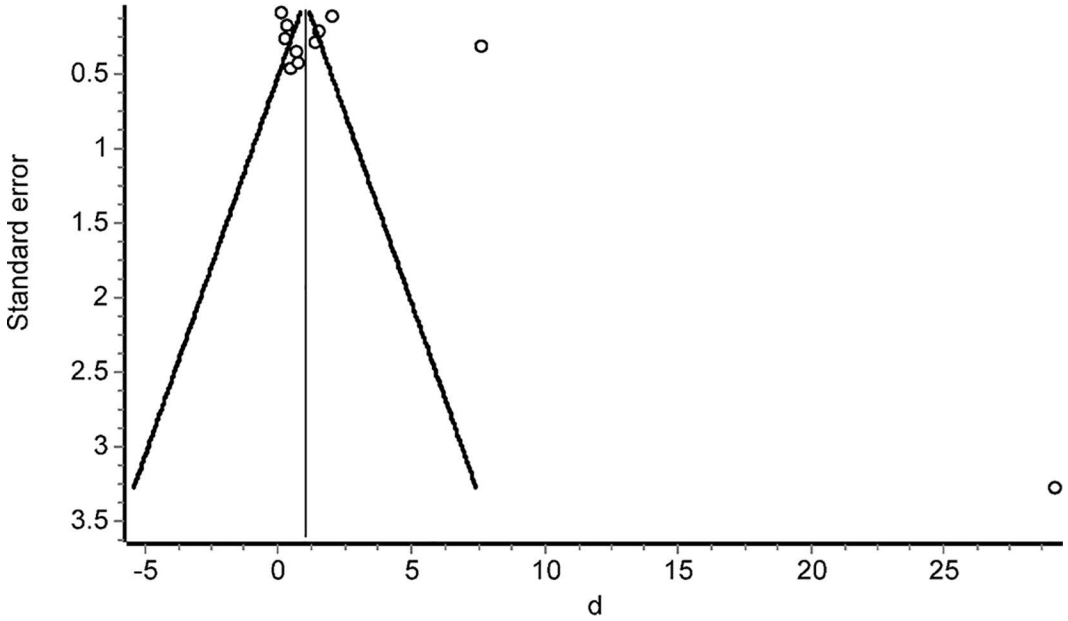

Fig 6. Funnel plot of the difference of procalcitonin (PCT) levels between sepsis and non-sepsis groups (Cohen's d effect sizes).

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antibiotic therapy, particularly when clinical signs are absent or unclear. On the other hand, if the biomarker levels are under the cut-off values defined for septic processes, this information may suggest that an inflammatory non-infectious process is occurring. So, these tests are also useful to avoid unnecessary antibiotherapy, which may result in toxicity and development of antimicrobial resistance.

PCT is a 116-aminoacid prohormone of calcitonin, which is mainly produced by the C-cells of thyroid gland and participates in calcium metabolism [39]. PCT is also synthesised in other tissues, including liver, kidney, lung and adipose tissue, in response to endotoxins, cytokines and other mediators released during the infection period [40]. PCT blood levels are barely detectable in healthy individuals. However, in the presence of systemic bacterial infection or, in a lower scale, fungal infection, its levels suddenly undergo a dramatic increase, following the infection course and then quickly subside after the control of the septic process. There is strong clinical evidence that PCT allows differentiation between non-infectious systemic inflammatory response and microbiological infection by bacteria or fungi and several studies confirm its utility as a reliable means to guide antibiotic use in community-acquired pneumonia and sepsis in intensive care patients [11,12,41–46]. Some studies also suggest its usefulness in the diagnosis and prognosis of sepsis in burns patients [17–19,27,47] [S3 File], though some controversy still persists [16,28,48]. PCT is currently one of the most investigated biomarkers and has already been integrated in treatment algorithms for patients with lower respiratory airways infections [12] and for ICU patients [28].

The main finding of this meta-analysis is that most of the included studies indicate that PCT can be a simple and very useful biomarker for the early identification of sepsis in burn patients, when used in combination with relevant clinical examination and other biomarkers available (e.g. leukocytosis, C-reactive protein, MR-pro-adrenomedullin) [18,49–52]. In fact, the pooled information resulting from this work suggests the feasibility of PCT quantification in these patients, showing that an average cut-off of 1.5 ng/mL is a strong indicator for sepsis suspicion and therefore for the initiation of antibiotherapy.

In addition, this work demonstrated that overall pooled area under the SROC curve was 0.87, with a sensitivity of 0.77 and a specificity of 0.65. The area under the SROC curve and the sensitivity are in agreement with the results published by Ren *et al.* [13], which reported that the area under the SROC curve was 0.92, with a sensitivity of 0.74. On the other hand, the specificity reported in this work was lower (0.65 vs 0.88) and the publication bias was significant. Thus, the inclusion of four additional studies in this meta-analysis, including two pediatric studies, contribute to support and strengthen the evidence supporting the interest of PCT levels as a biomarker for the early diagnosis of sepsis in burn patients. The sensitivity analysis, performed by excluding one study at each turn, also confirmed the sepsis effect on PCT concentration in burn patients. Nevertheless, the inclusion of these pediatric studies may explain the lower specificity and the higher heterogeneity reported in our work, as a population with different physiologic characteristics was considered in the analysis.

This work also included a sub-group analysis, comparing sepsis and non-sepsis groups of the studies included. This analysis revealed that sepsis group showed a statistical significant increase in the PCT mean values, in comparison with non-sepsis group. It also indicated that both groups were highly heterogeneous, though this parameter was higher in the sepsis group. Moreover, no significant publication bias was registered between sepsis and non-sepsis groups. The increase of PCT levels in patients diagnosed with sepsis corroborates the potential usefulness of this prohormone in burn patients with sepsis.

However, some studies reported that PCT levels can temporarily increase in some patients postoperatively, even in the absence of infection [41]. This increase is minor and rapidly subsides, but it must obviously be taken into account. In addition, some previous studies did not

confirm that PCT levels may be helpful for the diagnosis of sepsis in burn patients [23,24,48], which may result from several factors, such as a small sample size, heterogeneity among patients included in the analysis, different criteria for sepsis diagnosis and different timings of sampling [25].

This work has some limitations that must be considered when interpreting the results. Only 12 studies were available for meta-analysis and the number of patients included was in general small and heterogeneous between the different studies. The cut-offs values, which ranged from 0.5 to 5 ng/mL, and the methods used to quantify the PCT concentration also diverged in these studies. The high heterogeneity of the studies is also a factor that may rise questions about the utility of this biomarker. The inclusion of two pediatric studies, as referred, may had a significant impact in this parameter. Another limitation relies on the origin of the data included in the analysis. In fact, only published studies written in English were considered, which may imply the exclusion of significant and important data obtained in unpublished studies and studies written in other languages.

Based on these studies, in the authors' opinion, PCT levels should be determined daily in burn patients at high risk of infection (large total body surface area [TBSA] burns, mechanical ventilation, comorbidities, etc.), and at least twice a week for the rest of the burn patients. However, further studies with significant number of patients and planned to reduce the variability of cut-off values, number of timepoints and methods to quantify PCT levels should be conducted, to better evaluate the interest of PCT as a biomarker for early diagnosis of sepsis in burn patients. Studies combining the determination of PCT levels and the evaluation of other potential biomarkers or other clinical evidence should also be done, as generally the single determination of one biomarker is not sufficient to predict or early diagnose the septic process.

Conclusion

This meta–analysis showed PCT may be considered as a biomarker with a strong diagnostic ability to discriminate between the septic and the non-septic burn patients. The overall sepsis effect is significant and the overall association between PCT levels and the occurrence of mortality is also significant. This work clearly encourages the serial and frequent measurement of PCT levels in clinical practice for the management of burn patients, in order to timely identify the susceptibility to sepsis and to initiate the antimicrobial therapy, improving the patients' outcomes

Supporting Information

S1 File. This is the document in Annex 1.
(DOCX)

S2 File. This is the file of Systematic Revision–H. Rao.
(PDF)

S3 File. This is the file of Systematic Revision–E. Mann.
(PDF)

S4 File. This is the file of PRISMA 2009 checklist—The Use of Procalcitonin (PCT) for Diagnosis of Sepsis in Burn Patients.
(DOC)

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Funding acquisition: LC.

Investigation: LC VA.

Methodology: LC VA.

Project administration: LC LA.

Resources: LC VA.

Software: LC VA LA.

Supervision: JAP.

Validation: LA.

Visualization: LC LA.

Writing – review & editing: LC LA JAP.

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Chapter 3 Procalcitonin for the early diagnosis of sepsis in burn patients: a retrospective study

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Procalcitonin for the early diagnosis of sepsis in burn patients: A retrospective study

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ABSTRACT

Background: The gold standard for sepsis diagnosis in burn patient still relies on microbiological cultures, which take 48–72 h to provide results, delaying the start of antimicrobial therapy. Thus, biomarkers allowing an earlier sepsis diagnosis in burn patients are needed.

Methods: This retrospective observational study included 150 burn patients with total burned surface area ≥15%. Clinical diagnosis of sepsis among these patients was done according to the American Burn Association criteria. Biomarker (procalcitonin, white blood cells and platelet countings, prothrombinemia, D-dimers, C-reactive protein, blood lactate and temperature) values were available for 48 patients without sepsis (2767 timepoints) and 102 patients with sepsis (652 timepoints). Quantitative variables were compared with Mann–Whitney tests and qualitative variables were compared with Pearson chi-square test. Effect size was measured by the probability of superiority. Receiver operating characteristic (ROC) curves evaluate capacity for sepsis diagnosis. Sensitivity, specificity, positive and negative predictive values were calculated for some cut-off values, including the best cut-off defined by the maximum of Youden index.

Results: Statistically significant differences between the groups of septic and non-septic patients, with medium to large effect size, were detected for all the biomarkers considered, except temperature. PCT was the biomarker with the largest AUC and effect size (AUC = 0.71). Analysis of the PCT ROC curve showed that 0.5 ng/mL cut-off presented highest sensitivity and lowest specificity, whereas 1.5 ng/mL cut-off was associated with lowest sensitivity and highest specificity.

Conclusion: Procalcitonin showed to be the best of the biomarkers studied for an early diagnosis of sepsis. Its use should be considered in antimicrobial stewardship programs in Burn Units.

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1. Introduction

Severe burns are considered a relevant public health issue as they affect patients' physical and mental health, having an important negative impact on their quality of life. The management of burns also represent significant costs for the healthcare sector, in particular due to prolonged hospitalization periods and care of disfiguring injuries [1]. Sepsis is a comorbidity commonly observed in severe burn patients and is the major cause of their death [2,3]. In fact, severe burns increase the susceptibility to sepsis, as they propitiate the development of infections, due to several factors including skin injury, necrosis, use of catheters and other invasive devices, and exposure to nosocomial flora [4,5]. According to a recent review the literature, the prevalence of sepsis in burn patients ranges from 8% to 42% and the associated mortality rate varies form 28% to 65%. [6], its values being naturally related to the severity of the process [7] and the promptitude of the diagnosis and the beginning of therapy, as in other causes of initial injury [8,9]. The arising of multidrug resistant microorganisms in the last years has contributed to an even worst scenario [10]. Thus, it is of critical importance to timely diagnose and treat septic episodes in these patients. However, the identification of sepsis causative microorganisms takes 2–4 days, which may delay the start of the specific antimicrobial treatment [11]. On the other hand, the clinical and laboratorial findings of sepsis are also present in other clinical conditions with a systemic inflammatory response (trauma, anaphylaxis, pancreatitis, hemorrhage, etc.) [12] which complicates the differential diagnosis [13]. In this context, the use of biomarkers has been advocated to improve clinicians' ability to detect sepsis early in order to start a timely and adequate antimicrobial therapy.

The ideal biomarker should be suitable for the early diagnosis of sepsis (either as a part of a routine screening exam or at the first sign of a suspect clinical sign); should follow the course of the infection and reflect the efficacy of the therapy, allowing for its monitoring and suspension; should be safe and easy to measure; should be cost effective to follow-up and consistent across gender and ethnic groups. Such a biomarker has not yet been discovered, but several ones are already in use and coupled with a sound clinical examination may in fact support clinicians on the decision to start, and stop, antimicrobial therapy.

Among over 170 biomarkers described in the literature in the last decades [14], procalcitonin (PCT) has emerged, not without some controversy [15], as one of the most useful and reliable [16–28]. PCT is the hormonally inactive 116-amino acid precursor of calcitonin, a hormone that is mainly secreted by the C-cells of thyroid gland that are involved in calcium metabolism [29]. PCT can also be synthesized in extrathyroid tissues in response to endotoxins and proinflammatory cytokines release during and infection, but also in non-infectious conditions with systemic inflammation (e.g. multiple trauma, drug adverse reactions, cardiogenic shock, etc.). PCT serum levels are very low in healthy individuals but these levels markedly increase up to 1000-fold within 2–4 h of sepsis onset [30], and then rapidly decline after successful antimicrobial therapy. Scientific evidence corroborated that this peptide has a good capacity to distinguish between systemic non-infectious inflammatory response and septic conditions caused by bacteria or fungi in patients with community-acquired pneumonia [16,17] and in septic patients in intensive care units [18,19,29]. Several reports also support the utility of PCT for the diagnosis of sepsis in burn patients [21–28], though other authors question this evidence [31,32]. The current study aims to contribute for the determination of the potential utility of PCT as a biomarker for the early diagnosis of sepsis in burn patients. For such a purpose, PCT levels were assessed in different periods during hospitalization of burn patients in a specialized burn care unit and its discriminatory power was compared against other commonly used biomarkers.

2. Material and methods

2.1. Patients

The sample under analysis was composed by burn patients with partial, deep partial and/or full thickness burns comprising 15% or more of total burn surface area (TBSA), admitted consecutively from January 2011 to December 2014 at Coimbra Burns Unit (CBU), a department of Coimbra Hospital and University Centre (CHUC), Portugal. Burn patient data were obtained retrospectively by consulting the hospital database.

The diagnosis of sepsis was based on the American Burn Society (ABA) criteria [33]: a clinical suspicion of infection coupled with the presence of the presence of three or more of the following parameters: temperature >39 °C or <36.5 °C; tachycardia >110 beats per min; tachypnea >25 breaths per minute or minute ventilation >12 L/min; thrombocytopenia <100,000/μL; hyperglycemia (untreated plasma glucose >200 mg/dL or intravenous glucose use >7 U/h over 24 h; enteral feeding intolerance: abdominal distension or gastric residuals more than two times feeding rate or diarrhea >2,500 mL/min.

A timepoint was defined as day of analysis results; each patient had several timepoints. The total timepoints were distributed in two groups, sepsis and non-sepsis, according to the ABA criteria above. In order to avoid bias, subjectivity and trying to give more strength to the analysis, only microbiological blood tests were considered, independently of the known or suspected primary focus of the septic episode. From all patients of the sample, at least 3 (three) blood samples per week, in different days, were collected for PCT assessment. When there was a clinical diagnosis of sepsis according to ABA criteria, PCT was evaluated daily. In the cases where for the same patient, by any reason, there were two assessments of PCT in the same day, the highest value was taken for study purpose.

2.2. Laboratory measurements

At each time point, the following data were collected from the database: PCT, white blood cell counting, platelet counting, prothrombinemia, D-dimers, C-reactive protein (CRP), blood lactate and temperature.

PCT was measured with TRACE (time-resolved amplified cryptate emission) technology (Kryptor® PCT; Brahms AG; Hennigsdorf, Germany). White blood cells and platelets automated counting was performed by flow cytometry (UniCel DxH® 800 Coulter Cellular Analysis System; Beckman Coulter Ireland Inc.; Galway, Ireland). Prothrombin and D-dimers were quantified by automated latex enhanced immunoassay in human citrated plasma on the (ACL TOP® Family Systems, HemosIL D-Dimer HS 500; Instrumentation Laboratory SpA; Milano, Italy). CRP concentrations were quantitatively determined by the immunoturbidimetric method (Architect® c8000 System; Abbott; Wiesbaden, Germany).

2.3. *Statistical analysis*

Data were summarized by location measures (mean, median, minimum, maximum, percentiles) and dispersion measures (standard error [SE] and range).

The variables under study presented a non-Gaussian distribution. Under a nonparametric approach, the quantitative variables were analyzed with Mann–Whitney U test and qualitative variables were analyzed with Pearson chi-square test. To measure the effect size the probability of superiority (PS) was used. PS ranges from 0 to 1 and PS=0.5 states that there are no differences between the groups, meanwhile PS=0 or PS=1 state the maximum effect.

Receiver operating characteristic (ROC) curves, in particular the area under the curve (AUC), were performed to evaluate the selected biomarkers potential for sepsis diagnosis. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) were calculated for some cut-off values. The most accurate cut-off value was calculated by the Youden Index (J=sensitivity+specificity−1) [34].

Statistical analysis was performed with SPSS© 23.0 IBM© for Windows© (IBM Corp., Armonk, NY, USA). The statistical hypothesis tests with p-value <0.05 were considered significant. The confidence intervals are reported with 95% confidence level.

3. Results

This retrospective study included 150 patients with a total of 3,419 time points (2,767 time points without sepsis and 652 with sepsis). Table 1 presents the characteristics of study population, dividing the patients in two groups: sepsis group (patients with at least one time point with sepsis during hospitalization, n=102), and non-sepsis group (patients without any time point identified by sepsis during hospitalization, n=48). The sepsis and non-sepsis groups presented gender and age homogeneity. All other variables showed statistically significant differences between both groups.

Following the works of Lavrentieva et al. [26], a sub-analysis was done, reporting the median values of some biomarkers, sorting out the patients with less or more than 60% TBSA (Table 2). Patients with TBSA ≥60% presented significantly higher concentrations of PCT and D-dimers than patients with TBSA <60%, whereas prothrombin levels were higher in patients with TBSA <60%. Regarding the other biomarkers, no significant differences were observed between groups. Although significant, the effect sizes for all biomarkers and both TBSA groups were small.

In order to study the diagnostic power of the different biomarkers used at Coimbra Burns Unit, we compared biomarkers between septic and non-septic patients (Table 3). There were significant differences between the two groups of patients, with medium to large size effects, except for temperature. PCT was found to be the biomarker with the strongest effect size (0.28, a large effect size). Fig. 1 presents the median PCT values registered in burn patients with or without sepsis.

The areas under the ROC curves (AUC) were calculated to evaluate the biomarker power to differentiate a sepsis from a non-sepsis situation (Table 4). Temperature showed a low, not significant, accuracy for sepsis diagnosis. All the other biomarkers presented statistical significance (as the confidence intervals did not overlap) and accuracy (AUC different from 0.5; p-value<0.05).

Table 1 – Characteristics of the study population.

Characteristics	Sepsis[b]	Non sepsis	p-Value
Number of patients	102	48	
Gender (male/female)	57/45	29/19	0.600
Age (years)[a]	60.0 (41.5-79.0)	57.5 (40.8-64.8)	0.144
Burn degree (2nd/2nd & 3rd/3rd)	9/75/18	15/27/6	0.002*
ABSI score	8.0 (7.5-10.0)	6.0 (5.3-8.0)	0.000*
TBSA (%)[a]	29.0 (20.0-38.8)	19.0 (16.0-25.0)	0.000*
Inhalation injury (yes/no)	42/60	6/42	0.000*
Mechanical ventilation (yes/no)	54/47	8/40	0.000*
Antimicrobial therapy (days)[a]	16.0 (5.5-28.0)	0(0-6.0)	0.000*
Length of stay (days)[a]	33.0 (20.5-53.5)	17.0 (12.0-21.0)	0.000*
Mortality (yes/no)	33/69	5/43	0.004*
Surgery (number of interventions)[a]	4.0 (2.0-5.0)	1.0(0-2.0)	0.000*

[a] Values are median (Q1–Q3).
[b] At least one time point identified by sepsis during hospitalization.
* p-Values<0.05.

Table 2 – Biomarkers levels according to TBSA.

Biomarker	TBSA < 60%		TBSA ≥ 60%		p-Value	PS
	Nr. of timepoints	Median (Q1–Q3)	Nr. of timepoints	Median (Q1–Q3)		
Procalcitonin (ng/mL)	3056	0.39 (0.16–1.17)	286	0.62 (0.17–1.83)	0.036*	0.47
White cells (10^9/L)	3542	11,400 (8800–15,200)	305	13,200 (9550–19,050)	0.087	0.47
Platelets (10^9/L)	3539	279 (162–415)	305	304 (180–403)	0.442	0.49
Prothrombinemia (%)	3353	70 (61–78)	292	64 (58–71)	0.000*	0.40
D-dimers (μg/mL UEF)	2768	1.39 (0.93–2.16)	249	2.18 (1.12–4.06)	0.010*	0.46
C-react. prot. (mg/mL)	480	13.87 (8.84–20.65)	29	15.08 (8.58–25.96)	0.565	0.47
Lactate (mmol/L)	803	1.49 (1.01–2.06)	98	1.40 (1.06–2.11)	0.526	0.48
Temperature (°C)	4063	37.6 (37.0–38.3)	311	38.1 (37.1–38.7)	0.271	0.48

PS: probability of superiority.
* Statistically significant ($p < 0.05$).

Using all timepoints and assuming that PCT was the biomarker with the greatest AUC (Table 4 and Fig. 2) and that PCT demonstrated a large effect size with AUC >0.71 (according to Rice and Harris [35]), we analyzed the ROC curve of PCT to assess the most suitable cut-off for the diagnosis of sepsis in burn patients (Table 5). The cut-off of 0.5 ng/mL presented the highest sensitivity and the lowest specificity, whereas 1.5 was least sensitive and the most specific.

4. Discussion

We analyzed some putative biomarkers for early sepsis diagnosis in groups of septic and non-septic burn patients and concluded that PCT was the most reliable biomarker for sepsis diagnosis, in comparison to the other studied biomarkers (white cells and platelets counting, prothrombin, D-dimers, CRP, blood lactate and temperature). To our knowledge, this is the largest study of the use of PCT for sepsis diagnosis in burn patients, which allows a strong statistical analysis.

Currently, the leading cause of mortality of burn patients is sepsis. To prevent this mortality, it is crucial to start an early and adequate antimicrobial therapy because any delay increases the risk of death. However, it is not easy to correctly identify the sepsis onset in burn patients only based on clinical criteria, even when adjusted for this group of patients [23], because large burns induce an overwhelming systemic inflammatory response that mimics a septic episode. On the other hand, the superfluous prescription of antimicrobials must be avoided in order to decrease antimicrobial pressure and emergence of antimicrobial resistance. Attending that the gold standard for sepsis diagnosis is still the identification of microorganisms in the bloodstream and that in most health facilities blood cultures results take 48–72 h from the sampling, the use of biomarkers, alone [11] or in combination [36], has been regarded as an extremely important strategy to support clinician's decision to start antimicrobial therapy.

The use of PCT for sepsis diagnosis in burn patients has been first advocated by von Heimburg in 1998 [21], and supported later by several authors [22–28], but there are also some contradictory reports [31]. Our results corroborate the usefulness of PCT assessments in burn patients to early diagnosis sepsis and start antimicrobial therapy. Moreover, sample stratification by TBSA under and above 60% shows small, but significant, differences for PCT, prothrombin and D-dimers. P-value was higher for PCT suggesting a lesser correlation with burn size.

All the studied biomarkers, except temperature, showed statistically significant differences between septic and non-septic patients, but PCT stands out with the largest size effect. This superior diagnostic power is corroborated by the analysis of ROC curves, as PCT had the largest AUC.

Table 3 – Biomarkers levels in septic and non-septic burn patients.

Biomarker	Sepsis		Non sepsis		p-Value	PS
	Nr. of timepoints	Median (Q1–Q3)	Nr. of timepoints	Median (Q1–Q3)		
Procalcitonin	652	1.08 (0.40–3.94)	2767	0.32 (0.14–0.88)	0.000*	0.28
White cells	710	14.7 (11.5–19.6)	3218	10.9 (8.4–14.4)	0.000*	0.31
Platelets	709	182 (94–318)	3216	300 (189–421)	0.000*	0.33
Prothrombinemia	680	63 (54–70)	3044	70 (62–79)	0.000*	0.34
D-dimers	601	1.96 (124–371)	2487	1.31 (0.89–2.03)	0.000*	0.34
C-react. protein	102	16.47 (12.94–23.03)	407	13.14 (8.19–19.90)	0.000*	0.36
Lactate	294	1.80 (1.21–2.57)	607	1.35 (0.94–1.86)	0.000*	0.35
Temperature	730	37.5 (36.9–38.4)	3726	37.6 (37.0–38,3)	0.693	0.50

* Statistically significant ($p < 0.05$).

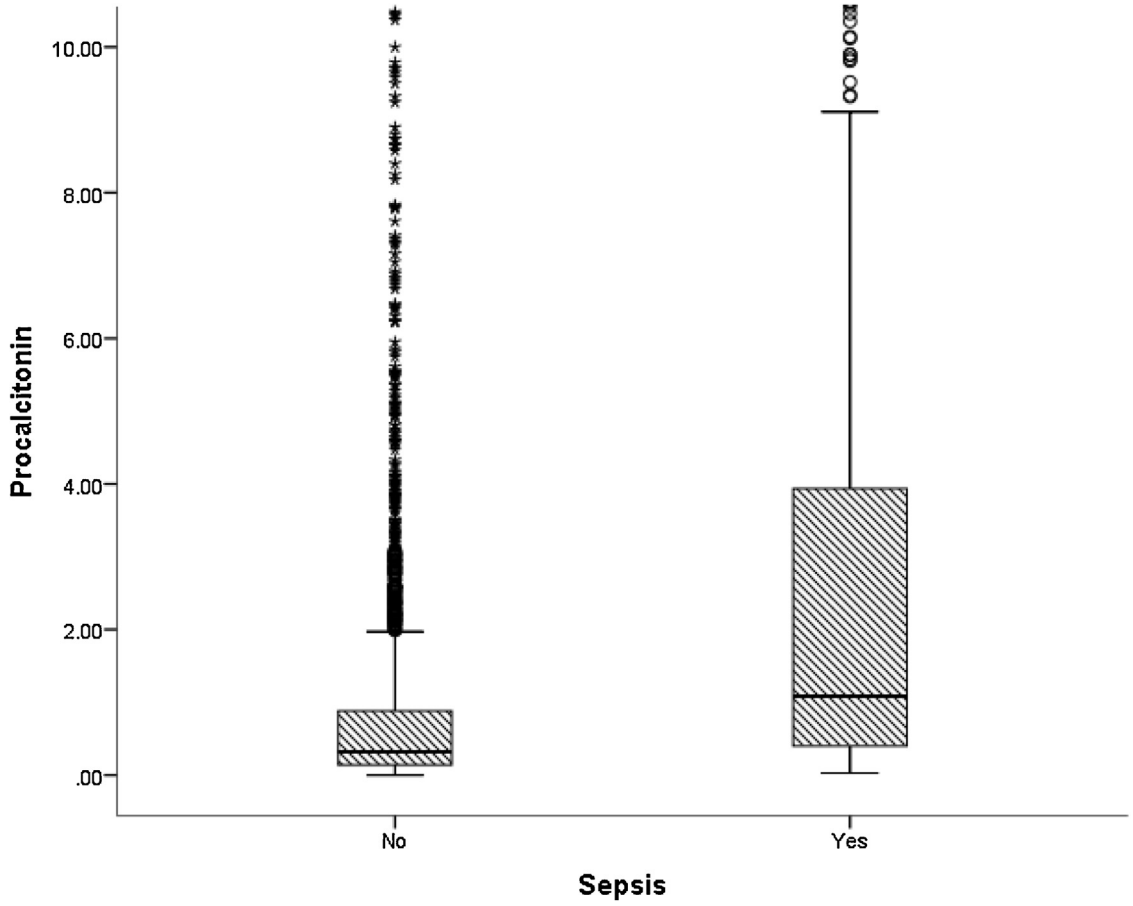

Fig. 1 – Median procalcitonin (PCT) values observed in septic (Yes) and non-septic (No) burn patients. In the presence present of sepsis, PCT levels are significantly higher.

Due to the variability of patient's characteristics and comorbidities, burn size, surgical procedures, analytic devices and other factors that may slightly influence PCT levels, a unique and absolute value for its cut-off for sepsis diagnosis cannot be definitively established, although several have been proposed. However, most authors agree that PCT dynamics is the most useful and reliable [37]. In this study sample, a PCT cut-off of 0.5 ng/mL demonstrated high sensitivity (0.71) but low specificity (0.62), whereas 1.5 ng/mL reached the highest specificity (0.83) but at the cost of a low sensitivity (0.43). The arbitrary choice of 1.0 ng/mL for cut-off may be advised attending to the better combination of sensitivity (0.52) and specificity (0.77), having a PPV of 38% and a NPV of 86%. However, the best approach may be to consider 0.5 ng/mL as an alert cut-off, which indicates the need of at least a daily measurement to monitor PCT evolution, and 1,0 or 1,5 ng/mL for the onset of pre-emptive antimicrobial therapy, de-escalating to the lower spectrum drug when antimicrobial sensitivity test is available.

The use of PCT in combination with other laboratorial and clinical sepsis biomarkers will certainly reinforce the diagnostic power [36]. Unfortunately, recent and promising

Table 4 – Biomarkers AUCs for sepsis diagnosis in burn patients.

Biomarker	AUC	SE	p-Value	Asymptotic 95% confidence interval		Timepoints
				Lower bound	Upper bound	
Procalcitonin	0.717	0.011	0.000*	0.696	0.738	3419
White cells	0.686	0.012	0.000*	0.663	0.709	3928
Platelets	0.672	0.012	0.000*	0.648	0.696	3925
Prothrombinemia	0.661	0.011	0.000*	0.639	0.684	3724
D-dimers	0.664	0.012	0.000*	0.640	0.688	3088
C-reactive protein	0.636	0.028	0.000*	0.581	0.692	509
Lactate	0.649	0.020	0.000*	0.610	0.688	901
Temperature	0.505	0.013	0.693	0.480	0.529	4456

* Statistically significant ($p < 0.05$).

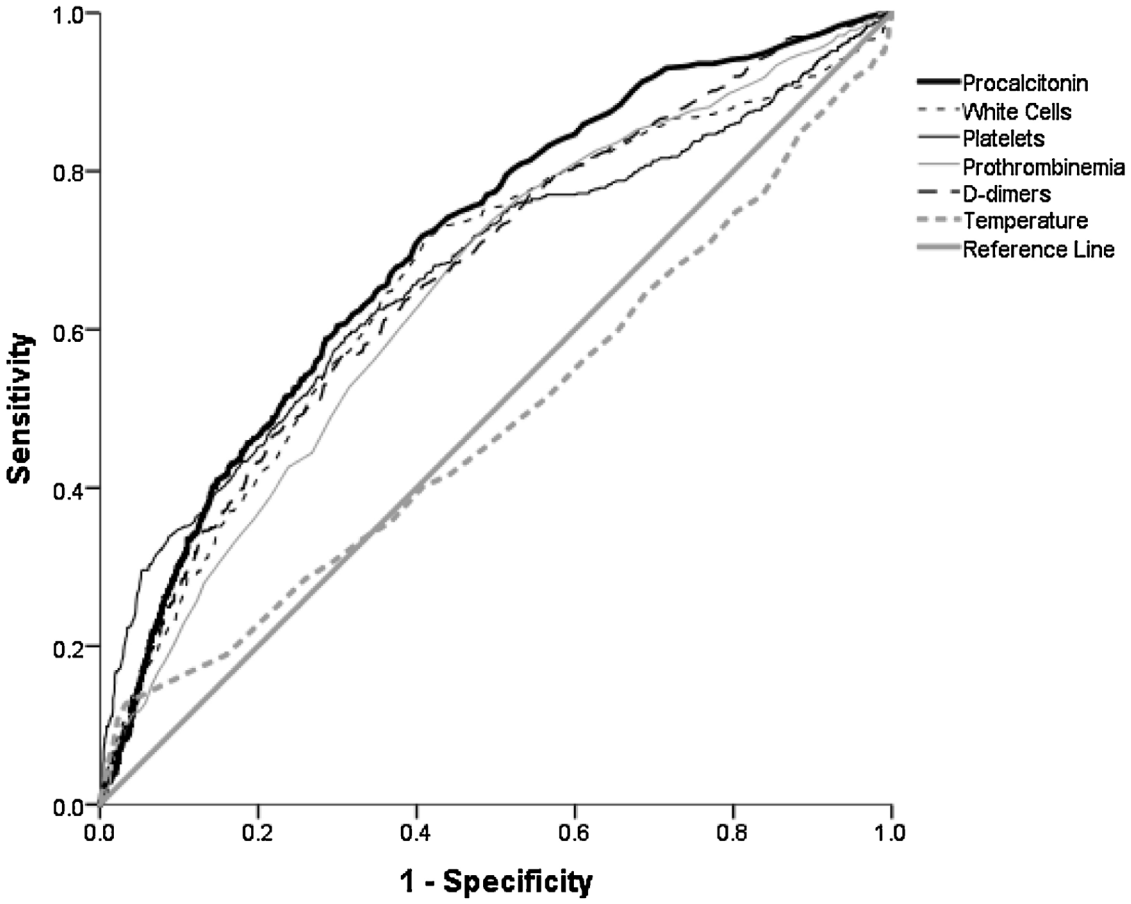

Fig. 2 – Receiver operating characteristic (ROC) curves for the biomarkers used for early diagnosis of sepsis in burn patients. Procalcitonin (PCT) showed the largest area under the ROC curve (AUC).

biomarkers, such as proadrenomedullin (MR-proADM) [38–40], CD64 neutrophil expression [41,42], biphasic transmittance waveform of activated partial thromboplastin [43,44], brain natriuretic peptide [45,46], presepsin [47–49], or extravascular ling water index [50] are not readily available in most of the hospitals, are expensive or are not yet validated for use in burn patients. Thus, in face of a suspected inflammatory systemic response, the use of clinical and traditional laboratorial biomarkers in combination with PCT [51,52], in a dynamic approach [51] with serial sampling [30], may represent a good alternative and strengthens the reliability of PCT diagnostic capacity.

Notwithstanding our interesting findings, this study has some limitations. Firstly, this is a retrospective and monocentric study, though analyzing a relatively large sample and a great number of time points with standard procedures for collection and recording of data. By the other hand, the methodology employed may raise some concern about sampling bias because a number of consecutive timepoints were included. However, as they were taken separately, the timepoints of patients from sepsis and non-sepsis groups being undoubtedly distinct can, at least in great part, avoid bias suspicion.

One strength of the study is the use of well-defined and internationally accepted criteria for clinical suspicion of sepsis [33], allowing a sound statistical analysis. Similar studies should be developed in other burn centers and new biomarkers should also be assessed. Additionally, a well-designed

Table 5 – Sensitivity and specificity of PCT cut-offs for the diagnosis of sepsis in burn patients.

Cut-off (ng/mL)	Sensitivity	Specificity	Youden index[a]	PPV	NPV
0.5	0.71	0.62	0.33	0.87	0.34
1.0	0.52	0.77	0.29	0.86	0.38
1.5	0.43	0.83	0.27	0.86	0.38

NPV: negative predictive value.
PPV: positive predictive value.
[a] Youden index (J = sensitivity + specificity − 1).

international multicentric prospective study, comparing PCT to other biomarkers, would certainly be very valuable for the complete understanding its potential for the diagnosis of sepsis in burn patients.

5. Conclusions

This study shows that PCT monitoring can help the early sepsis diagnosis and support clinicians on the decision to start or stop antimicrobial therapy in burn patients. Thus, as proposed by Meisner [30] for ICU patients, PCT measurements in antimicrobial stewardship programs in Burn Units may as well be an important tool to an early sepsis diagnosis and to potentially reduce mortality, toxicity, antimicrobial resistance arousal and financial burden.

Conflict of interests statement

The authors declare that no competing interests exist.

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Chapter 4 Checking procalcitonin (PCT) suitability for prognosis and antimicrobial therapy monitoring in burn patients

Luis Cabral, Vera Afreixo, Rita Meireles, Miguel Vaz, Catarina Chaves, Marisa Caetano, Luís Almeida, José Artur Paiva

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Checking procalcitonin suitability for prognosis and antimicrobial therapy monitoring in burn patients

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Abstract

Background: Due to greater infection susceptibility, sepsis is the main cause of death in burn patients. Quick diagnosis and patient stratification, early and appropriated antimicrobial therapy, and focus control are crucial for patients' survival. On the other hand, superfluous extension of therapy is associated with adverse events and arousal of microbial resistance. The use of biomarkers, necessarily coupled with close clinical examination, may predict outcomes, stratifying patients who need more intensive care, and monitor the efficacy of antimicrobial therapy, allowing faster de-escalation or stop, reducing the development of resistance and possibly the financial burden, without increasing mortality. The aim of this work is to check the suitability of procalcitonin (PCT) to fulfill these goals in a large sample of septic burn patients.

Methods: One hundred and one patients, with 15% or more of total body surface area (TBSA) burned, admitted from January 2011 to December 2014 at Coimbra Burns Unit (CBU), in Portugal were included in the sample. All patients had a diagnosis of sepsis, according to the American Burn Association (ABA) criteria. The sample was factored by survival (68 survivors and 33 non-survivors). The maximum value of PCT in each day was used for statistical analysis. Data were summarized by location measures (mean, median, minimum, maximum, quartiles) and dispersion measures (standard error and range measures). Statistical analysis was performed with SPSS© 23.0 IBM© for Windows©.

Results: There were statistically significant differences between PCT levels of patients from the survivor and non-survivor groups during the first and the last weeks of hospitalization as well as during the first week after sepsis suspicion, being slightly higher during this period. During the first 7 days of antimicrobial therapy, PCT was always higher in the non-survivor, still without reaching statistical significance, but when the analysis was extended till the 15th day, PCT increased significantly, rapidly, and steadily, denouncing therapy failure.

Conclusion: Despite being not an ideal biomarker, PCT proved to have good prognostic power in septic burn patients, paralleling the evolution of the infectious process and reflecting the efficacy of antimicrobial therapy, and the inclusion of its serial dosing may be advised to reinforce antimicrobial stewardship programs at burn units; meanwhile, more accurate approaches are not available.

Keywords: Burns, Sepsis, Procalcitonin, Prognosis, Antimicrobial stewardship

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Background

Sepsis is still nowadays the main cause of death in burn patients due to the impact of extensive burns in all organ systems, affecting homeostatic mechanisms, and to the greater susceptibility of this population to infection [1, 2], related to the loss of the cutaneous barrier, immunosuppression, use of invasive devices, nosocomial flora, etc. Survival is directly dependent on the institution of prompt and adequate antimicrobial therapy [3]. However, the gold standard for sepsis diagnosis still relies on the identification of microorganisms in blood cultures, which unfortunately are positive only in 20–30% of all confirmed bloodstream infections, and their results may take 48 to 72 h to reach the prescriber [4]. While more rapid methods of microbiological identification, such as polymerase-chain reaction (PCR) [5], matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), gene expression profiling, aptamer panels, etc. [6], are not either widely available or fully developed, the use of early empirical often broad-spectrum antibiotic therapy is warranted. This empirical strategy increases the likelihood of cure of infection and survival but negatively impacts in terms of microbiome, leading to the selection and emergence of antimicrobial resistance. In this context, biochemical biomarkers, namely procalcitonin (PCT) alone [7, 8] or integrating a composite panel [9–12], and always coupled with thorough clinical examination, may be an important aid for the early suspicion of sepsis and rapid institution of therapy, which is strongly associated with improved outcomes [13, 14].

Table 1 Patients' characteristics

Characteristics	Survivors	Non-survivors	*p* value
Number of patients	68	33	
Age (years)	53.0 ± 2.4 (18–85)	70.5 ± 3.5 (28–90)	0.000*
Male, gender (%)	38 (55.9%)	19 (57.6%)	0.872
Burn degree (2nd/2nd and 3rd/3rd)	8/50/10	1/24/8	0.219
ABSI score	8.0 ± 0.2 (4–13)	10.4 ± 0.4 (8–17)	0.000*
TBSA burned (%)	28.2 ± 1.6 (14–75)	40.7 ± 3.6 (15–90)	0.000*
Inhalation injury (%)	45 (66.2%)	15 (45.5%)	0.047*
Mechanical ventilation (%)	36 (52.9%)	4 (12.1%)	0.000*
Days of mechanical ventilation	11.3 ± 2.3 (0–70)	22.4 ± 3.8 (0–76)	0.000*
Duration of sepsis episode (days)	5.5 ± 0.6 (1–24)	10.6 ± 1.8 (1–43)	0.005*
Antimicrobial therapy (days)	20.8 ± 2.4 (0–104)	18.5 ± 3.4 (0–64)	0.374
Number of surgical interventions	4.3 ± 0.3 (0–15)	2.5 ± 0.6 (0–12)	0.000*
Length of stay (days)	43.1 ± 3.2 (8–180)	29.9 ± 5.0 (3–113)	0.001*

Values are mean ± S.E. (min-max)
*Significant difference at *p* value < 0.05
ABSI Abbreviated Burn Severity Index, *TBSA* Total body surface area, *S.E.* Standard error

Table 2 Analysis of individual procalcitonin (PCT) location measures in survivor and non-survivor patients, showing statistically significant differences for all parameters

	Survivors	Non-survivors	*p* value
PCT minimum	0.10 ± 0.01 (0.02–0.39)	2.84 ± 1.59 (0.06–48.39)	0.000*
PCT median	0.57 ± 0.10 (0.05–4.31)	4.73 ± 1.93 (0.27–58.99)	0.000*
PCT mean	2.04 ± 0.48 (0.05–26.28)	7.00 ± 1.98 (0.05–58.99)	0.000*
PCT maximum	18.40 ± 4.38 (0.07–237.60)	28.07 ± 5.98 (0.87–145.40)	0.002*

Values are mean ± S.E. (min-max)
*Significant difference at *p* value < 0.05
PCT procalcitonin, *S.E.* Standard error

PCT is a 116-amino acid precursor of calcitonin, which synthesis and secretion, encoded by first calcitonin gene (CALC-I gene), and normally restricted to thyroid C cells and some neuroendrocrine cells of the lungs and gut, is upregulated by the presence in the blood of microbial toxins, necrotic body cells, and some proinflammatory cytokines (IL-1, IL-6, TNF-α, etc.), in a synergistic way, starting to be produced in great amounts by many other nonendocrine types of cells, including monocytes and adipocytes [15], reaching measurable levels in 2–4 h after onset of the infectious process, peaking at 24–30 h, and rapidly subsiding with recovery. PCT increment is less pronounced with fungal infection and is absent in viral disease, allegedly due to inhibition of its secretion by some cytokines released as a response to viral infection, like interferon-γ [16].

Besides its utility to help clinicians in the diagnosis of sepsis [17] including patients admitted to burn units [18], the magnitude and duration of PCT elevation seems to correlate with injury severity and outcome, and there are several published works analyzing its potential for the prognosis and for the monitoring of antimicrobial therapy, helping decisions on early antibiotic de-escalation or rescue therapy [19–21]. Most of these are focused in lower respiratory tract infections and/or intensive care patients, while papers on septic burn patients are scarce [22].

The purpose of this work is to evaluate the feasibility of PCT use to predict the outcome and to monitor the efficacy of antimicrobial therapy in a sample of severe adult burn patients.

Methods

The sample under analysis was composed by 101 burn patients, with 15% or more of total body surface area (TBSA) burned, admitted from January 2011 to

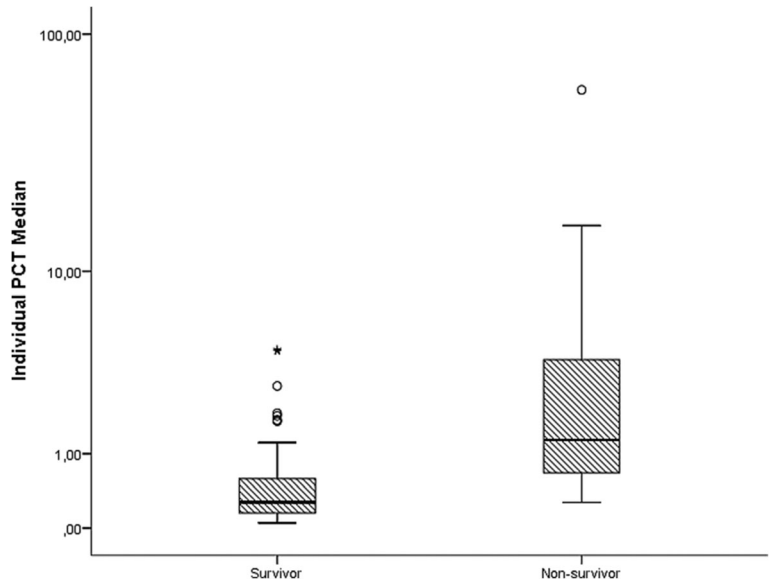

Fig. 1 Box plots of individual procalcitonin (PCT) median according to survivor and non-survivor groups. *$p < 0.05$ means significant differences

December 2014 at Coimbra Burns Unit (CBU), a department of Coimbra Hospital and University Center (CHUC), in Portugal. Being a retrospective observational study of patients from a suitably anonymized dataset, involving only recording data from the medical record, the Ethics Committee from CHUC, according to the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethics Guidelines, waived the need of informed consent.

All the patients had a diagnosis of sepsis. This diagnosis was done according to the American Burn Association (ABA) criteria [23]: a clinical suspicion of infection coupled with the presence of three or more of the following parameters: temperature > 39 or < 36.5 °C; tachycardia > 110 beats per minute; tachypnea > 25 breaths per minute or minute ventilation > 12 L/min; thrombocytopenia < 100,000/μL; hyperglycemia (untreated plasma glucose > 200 mg/dL or intravenous glucose requirement > 7 U/h over 24 h); and enteral feeding intolerance: abdominal distension or gastric residuals more than two times feeding rate or diarrhea > 2500 mL/24 h.

PCT was measured with time-resolved amplified cryptate emission (TRACE) technology (Kryptor PCT; Brahms AG; Hennigsdorf, Germany). The sample was factored by survival (68 survivors and 33 non-survivors). The maximum value of PCT in each day of the study was used for statistical analysis and when samples were not collected in some days (till a maximum of 5 days), the missing values of the interval were calculated as the median value between the PCT determinations available.

Statistical analysis

Data were summarized by location measures (mean, median, minimum, maximum, quartiles) and dispersion measures (standard error and range measures).

Table 3 Evolution of procalcitonin levels during the first week of hospitalization for survivor and non-survivor groups

First week of hospitalization						
	Survivors			Non-survivors		
Day	*N*	Median	Q1–Q3	*N*	Median	Q1–Q3
1	58	0.290	0.150–1.160	27	1.6600	0.405–7.995
2	65	0.345	0.170–1.650	28	2.0550	0.270–6.840
3	66	0.360	0.170–1.640	27	1.9800	0.565–3.220
4	67	0.420	0.175–1.155	26	2.0550	0.520–4.170
5	67	0.345	0.160–0.830	26	1.7900	0.700–4.370
6	68	0.330	0.155–0.785	25	1.3100	0.560–2.850
7	68	0.360	0.160–0.985	24	1.7000	0.730–5.555

Q1-Q3 1st Quartile- 3rd Quartile

Table 4 Evolution of procalcitonin levels in the last week of hospitalization for survivor and non-survivor groups

Last week of hospitalization						
	Survivors			Non-survivors		
Day	*N*	Median	Q1–Q3	*N*	Median	Q1–Q3
1	67	0.180	0.100–0.395	22	1.050	0.700–2.370
2	68	0.160	0.095–0.435	24	1.0150	0.435–2.830
3	68	0.150	0.080–0.400	26	1.1100	0.560–2.510
4	68	0.160	0.080–0.320	26	1.2000	0.460–2.825
5	68	0.150	0.070–0.355	27	1.4700	0.650–3.570
6	68	0.140	0.070–0.360	28	2.3650	0.710–5.820
7	68	0.125	0.070–0.365	31	3.8200	1.100–10.235

Q1-Q3 1st Quartile- 3rd Quartile

The variables under study present a non-Gaussian distribution. Under a nonparametric approach, the quantitative variables were compared with the Mann-Whitney U tests and qualitative variables were compared with the Pearson chi-square test. Time variations of PCT levels were tested using Friedman's test and Kendall's W ranges from 0 (no agreement) to 1 (complete agreement).

To measure the difference effect size between the two independent groups, the probability of superiority (PS) was used. PS ranges from 0.0 to 1.0 and PS = 0.5 state that there are no differences between the groups [A] and PS = 0 or PS = 1 states the maximum effect.

Statistical analysis was performed with SPSS© 23.0 IBM© for Windows©, and in a statistical hypothesis test, a p value ≤ 0.05 means the effect was considered significant.

Results

Sample description

Population characteristics are described in Table 1. After factorization by survival, a significant heterogeneity was found between the two groups (68 survivors and 33 non-survivors) for the age of the patients, the Abbreviated Burn Severity Index (ABSI)

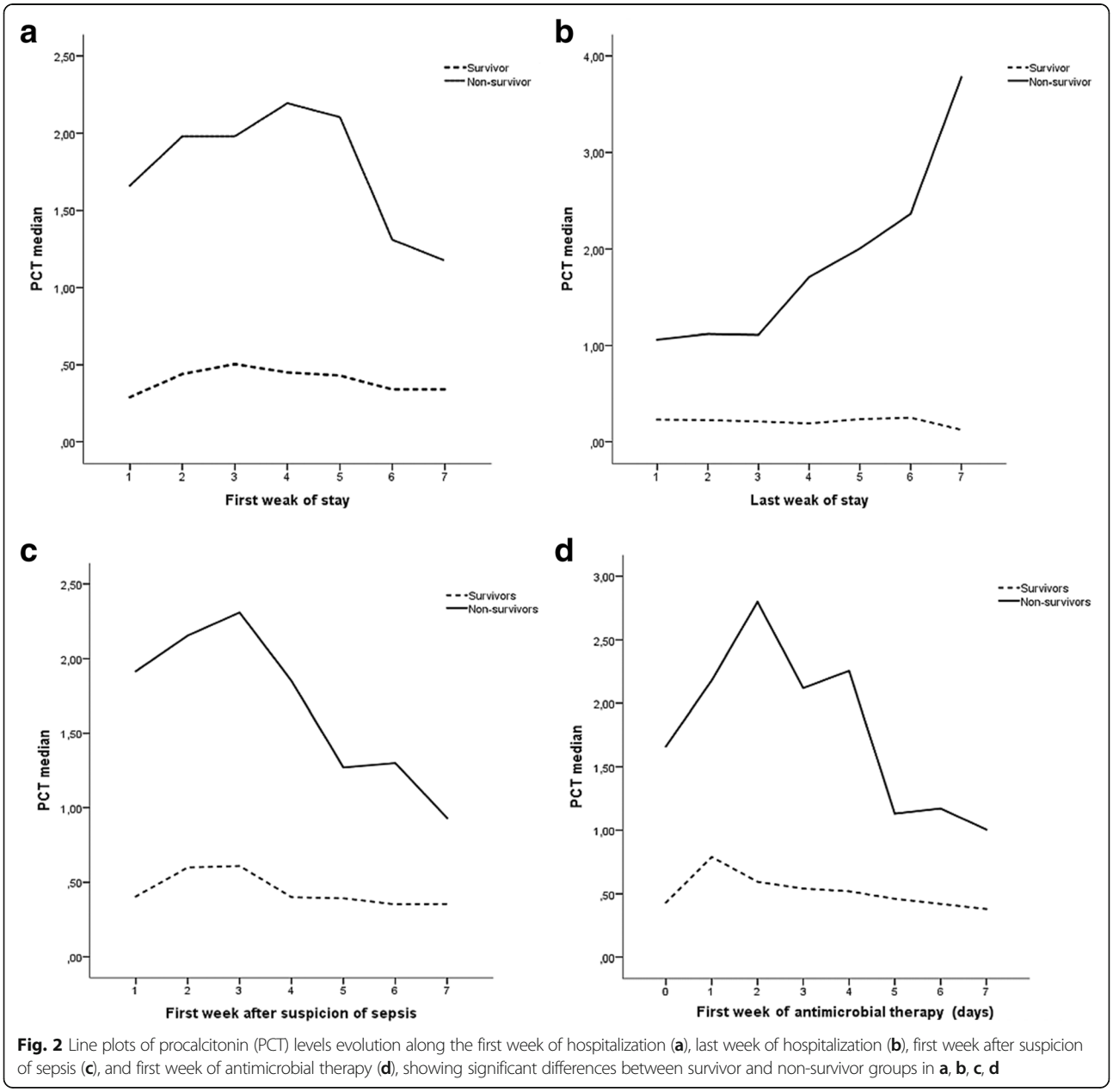

Fig. 2 Line plots of procalcitonin (PCT) levels evolution along the first week of hospitalization (**a**), last week of hospitalization (**b**), first week after suspicion of sepsis (**c**), and first week of antimicrobial therapy (**d**), showing significant differences between survivor and non-survivor groups in **a**, **b**, **c**, **d**

Table 5 Evolution of procalcitonin levels during the first week after suspicion of sepsis for survivor and non-survivor groups

First week after suspicion of sepsis						
	Survivors			Non-survivors		
Day	*N*	Median	Q1–Q3	*N*	Median	Q1–Q3
1	58	0.385	0.160–2.260	26	1.915	0.460–6.170
2	63	0.600	0.200–2.430	27	2.100	0.560–6.735
3	65	0.610	0.200–2.120	26	2.310	0.550–5.610
4	65	0.400	0.200–1.170	24	1.850	0.485–5.965
5	64	0.395	0.210–1.160	23	1.270	0.545–3.820
6	60	0.345	0.170–1.240	21	1.300	0.720–4.910
7	52	0.330	0.160–1.470	20	0.930	0.515–2.350

Q1-Q3 1st Quartile- 3rd Quartile

score (Additional file 1) [24], the TBSA burned, the presence of inhalation injury, the need of mechanical ventilation and its duration, the number of surgical interventions, the duration of sepsis episode, and the length of the stay at the burn unit. Heterogeneity was not found for gender, burn degree, and duration of antimicrobial therapy.

Table 2 shows the comparison of individual PCT location measures, presenting significant differences between survivors and non-survivors in all statistical parameters (minimum, median, mean, maximum).

The box plots of individual median PCT levels for each group are presented in Fig. 1, being significantly lower for survivors.

PCT evolution along the first week of stay

Table 3 shows the evolution PCT levels in patients from the survivor and non-survivor groups during the first week of stay at CBU. The data presents missing values of the PCT in some of the days of hospitalization and this is the reason for this variation in the number of individuals by scenario. Differences between PCT levels of patients from the survivor and non-survivor groups during the first week of hospitalization are statistically significant (Fig. 2a and Table 6).

PCT evolution along the last week of stay

The evolution of PCT levels for survivor and non-survivor groups in their last week of stay at CBU is presented in Table 4. A statistically significant difference was also demonstrated for this period of time (Fig. 2b and Table 6).

PCT evolution in the first week after suspicion of sepsis

A statistical analysis of PCT evolution in the first week after suspicion of sepsis, as defined by ABA criteria, was also carried out. Data are presented in Table 5. A significant difference between survivor and non-survivor groups was detected (Fig. 2c and Table 6).

In order to compare the relative prognostic value of PCT levels in each of the abovementioned periods (first week of hospitalization, last week of hospitalization, and first week after sepsis suspicion), statistical tests were done, namely Friedman test *p* value and Mann-Whitney U test *p* values with Sidak correction (Table 6).

Furthermore, the PS effect [25] was determined. The results are transcribed in Table 7.

PCT evolution with antimicrobial therapy

No statistically significant difference was found between the groups, but a within-group significant variation was detected, with a progressive decline along the first 7 days, supposedly due to antimicrobial action (Fig. 2d and Table 8). When the analysis was extended to the 15th day, it was found that PCT levels increased rapidly and steadily until the day of death in non-survivors, what did not happen in the survivor group, as seen in Fig. 3.

Discussion

Even acknowledging all advances in critical care, extensive burns are still associated with high morbidity and mortality mainly due to septic episodes [26, 27]. In the last years, diverse studies were published showing the utility of dosing PCT levels as an aid to the diagnosis of systemic infection in burn patients [28–34], particularly when a dynamic approach is used [35]. Notwithstanding the core decision should rely on the clinical features and never on a biomarker alone [36], PCT dosing may support the suspect of

Table 6 Comparison between survivors and non-survivors during three periods of stay (first week of stay, last week of stay, and first week after suspicion of sepsis)

Period	Survivors			Non-Survivors			Global difference
	p value[a]	Kendall's *W*	*N*	*p* value[a]	Kendall's *W*	*N*	*p* value[b]
First week of stay	0.925	0.006	58	0.504	0.042	21	0.000*
Last week of stay	0.000*	0.162	67	0.050	0.095	22	0.000*
First week after suspicion of sepsis	0.000*	0.117	46	0.217	0.077	18	0.002*

Significant difference (*p value < 0.05)
[a]Friedman test p value
[b]The minimum p value of all simultaneous Mann-Whitney U tests with Sidak correction

Table 7 Probability of superiority (PS) effect in procalcitonin levels due to mortality in different periods of stay (first week of stay, last week of stay, and first week after suspicion of sepsis)

PS effect	D1	D2	D3	D4	D5	D6	D7
First week of stay	0.29	0.26	0.25	0.25	0.21	0.24	0.25
Last week of stay	0.06	0.10	0.13	0.15	0.16	0.20	0.15
First weak after suspicion of sepsis	0.32	0.30	0.31	0.28	0.28	0.24	0.29

ongoing and uncontrolled systemic infection when its values keep rising, or at least does not subside in consecutive analysis, indicating that something must be done to control a probable septic process before it can lead to irreversible damage. Apart its potential to improve clinicians' diagnostic capacity, PCT has been used with success at the emergency departments [37, 38], to predict the prognosis of suspected septic patients and to stratify them according to the risk of death and the necessity of admission in intensive care units (ICU) [39–41]. PCT levels at admission and, much more reliable [42], its evolution on subsequent days may give insights on the ultimate outcome, which is crucial to clinical management and may be of great importance to inform patient's relatives and for judicial concerning [43–47]. This valuable predictive power was not found for C-reactive protein (CRP) or white blood cells counting, another currently employed blood biomarkers [48–53]. The prognostic power of PCT dosing has also been stated for burned patients by Kim et al. [54] who, in a prospective observational study with a cohort of 175 patients, showed a significant correlation between PCT levels and mortality rate. In this context, it is worth to note, as referred by Piroglu et al. [55], that clinical scoring systems used to predict mortality of intensive care patients, like Acute Physiology and Chronic Health Evaluation Score II (APACHE II), Simplified Acute Physiology Score (SAPS), Sequential Organ Failure Assessment (SOFA), and Pediatric Risk of Mortality (PRISM), do not include parameters specific for burn patients, and these authors showed that combination of the former score with PCT significantly increased its accuracy. A prospectively study of Lavrentieva et al. [31], including 145 patients, concluded that the maximum PCT level has prognostic value in burn patients, and Mokline et al. [32] found a close correlation of PCT levels with sepsis severity, showing that increasing values were linked with worse outcomes and vice versa.

Table 8 Evolution of procalcitonin levels in the first week of antimicrobial therapy for survivor and non-survivor groups

First week of antimicrobial therapy						
Day	Survivors			Non-survivors		
	N	Median	Q1–Q3	*N*	Median	Q1–Q3
1	66	0.6300	0.240–3.020	24	2.080	0.945–2.810
2	68	0.5500	0.225–2.475	24	2.680	0.870–5.155
3	66	0.4950	0.220–1.300	24	1.945	0.750–7.205
4	66	0.4400	0.220–1.140	23	2.010	0.940–4.485
5	65	0.3500	0.170–1.140	22	1.065	0.550–4.490
6	61	0.3700	0.170–1.250	21	1.070	0.380–3.270
7	59	0.3700	0.175–0.920	21	0.960	0.660–2.420

Q1-Q3 1st Quartile- 3rd Quartile

Another important use of PCT dosing is guiding antimicrobial therapy in septic ICU patients, which is becoming generally accepted [56], supported by several trials [57–62], systematic reviews, and meta-analysis [63–67]; however, some authors still consider that more studies on its safety and efficacy are needed yet [68, 69]. Once a clinical suspicion of sepsis is done, and in particular if corroborated by abnormally elevated PCT levels, empirical antimicrobial therapy, coupled with focus control when feasible, must be immediately started because survival is mostly depending on it and any delay, even hourly, is directly related with an increase in mortality [13, 70, 71]. On the other hand, there is an overwhelming acceptation that a lengthening of antimicrobial therapy beyond that strictly necessary to control the infectious process favors the development of microbial resistance, contributing to the soaring public health risk of having each time less sensitive microorganisms and lack of antimicrobials to combat them [72]. Many published works describe PCT kinetics as a mirror of the evolution of the infectious episode [73–75] as well as a trustable indicator of the antimicrobial therapy efficacy, allowing an early de-escalation and/or stopping of drug administration when its levels progress and consistently subside [76, 77]. When PCT levels keep elevated or even increasing, this is a sound indication that therapy is not working and/or that there are still infectious foci to clean, and if the situation is not rapidly controlled, a bad outcome is foreseeable.

Several authors have discussed in recent works this use of PCT, and a body of evidence is growing to support this approach. Jensen et al. in a trial (PASS Study) [78] published in 2011 advised against PCT-guided antimicrobial escalation, linking it to increased organ-related harm and length of stay at the ICU, without improvement in the outcomes. However, the sample analyzed came from just one developed country with antimicrobial restriction and a traditionally low microbial resistance. On the other hand, focus was not put on the possibility of using PCT levels to help decision on antibiotherapy discontinuation neither a subgroup analysis

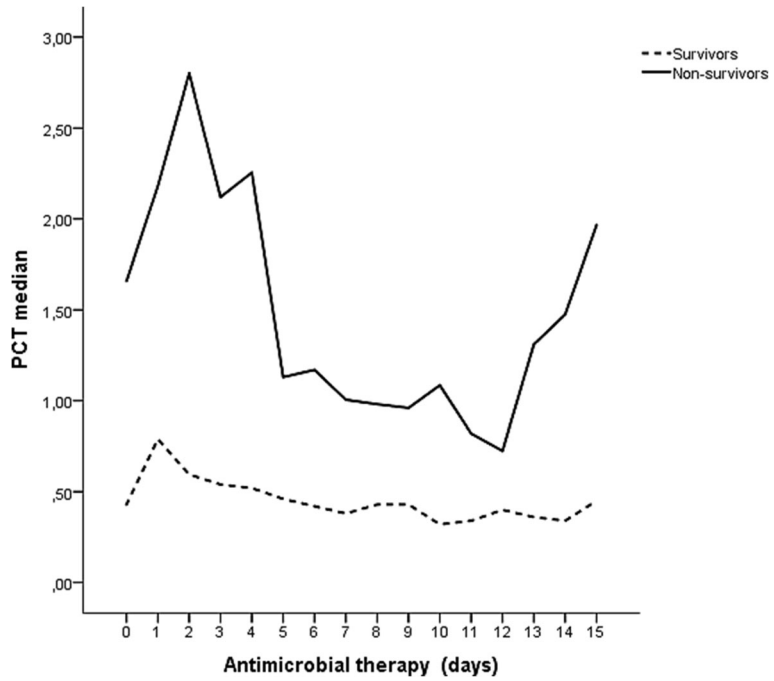

Fig. 3 Line plots of procalcitonin (PCT) evolution in the first 15 days of antimicrobial therapy

on burn patients was done. Nevertheless, and even if antimicrobial escalation may be somewhat controversial, PCT has proven to be very useful to monitor antimicrobials efficacy, with its levels paralleling clinical evolution, and to indicate when it is safe to stop it without prejudice to the patients [79]. Indeed, this methodology has proved to safely decrease antimicrobial consumption [80] by reducing days of antimicrobial therapy with strong potential to lower resistance development. This approach has already been validated for use in ICUs, with proven reduction of antimicrobial consumption without increase in morbidity or mortality [81]. Indeed, de Jong et al., in the largest prospective study in ICU patients published to date (SAPS Study) [61], were even able to show a significant reduction of mortality rate. The ever wider diffusion of PCT test, reducing its costs, and its efficacy in this setting, made also possible for some authors to consider it as probably cost-effective [82–86]. In a recent paper, Lavrentieva et al. [87] reported significantly shorter durations of antibiotic treatment in a PCT-guided group of burns patients compared to controls without differences in main outcome characteristics, including mortality rate, length of mechanical ventilation, and length of stay.

Among the limitations of this study are naturally its single-center, retrospective observational character as well as lacking of subgroup analysis according to concomitant pathologies. The definition of a precise *cut-off* of PCT levels for predicting outcomes or stopping antimicrobial therapy was also beyond the scope of this analysis and, as recognized in the literature, it will always be dependent on patient characteristics and facility features, and it is PCT kinetics that deserved authors attention, in spite of 100 ng/mL was often taken as an alert signal. On the positive aspects are the sample size and the strict use of ABA burn sepsis definitions for inclusion criteria. The strength of results from the present study would be largely enhanced by a desirable prospective multicentric trial.

The use of prognostic biomarkers in order to predict outcomes as well for guiding antimicrobial therapy in sepsis patients is nowadays a common practice in intensive care wards. As anytime more acknowledged in the literature, antimicrobial stewardship programs employing current available biomarkers or preferably, a panel of diverse ones, always associated with repeated clinical evaluation, may decisively improve patients' stratification and antimicrobial use, optimizing patients outcome, reducing the spread of microbial resistance, and cutting financial burden [88–93]. Meanwhile more sophisticated and individualized system-based (integrating genomics, metabolomics, and proteomics) [94–96] data are not available to more accurately predict outcomes and tailor treatment options for burn victims, as well as other intensive care patients, PCT dosing will remain one of the more useful tools to help clinicians decisions.

Conclusion

In spite of its limitations, the close correlation between PCT levels and patients' outcomes statistically demonstrated in the present work backs its use for prognosis determination in severe burn patients. Additionally, this study showed that the persistency of abnormally elevated PCT along the days of antimicrobial therapy was linked with poor outcomes in this set of patients, opposed to what happens when their levels fall in a consistent way, reflecting its efficacy.

Prospective multicentric studies would surely give more strength to the generalization of PCT use for prognosis and antimicrobial stewardship in burn patients and are much needed.

Additional file

Additional file 1: Annex 1. Abbreviated Burn Severity Index. (DOCX 19 kb)

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Availability of data and materials

The data that support the findings of this study are available from the datasets of the Informatics Department of Coimbra University Hospital Centre but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors, upon a reasonable request and after permission of the Ethics Committee from Coimbra University Hospital Centre.

Authors' contributions

LC and VA designed the study, interpreted the data, and draft the manuscript. VA was responsible for most of the statistical analysis. RM, MV, CC, and MC were responsible for the data acquisition, search of literature, and made suggestions for its integration along the manuscript. CC and MC made also substantial intellectual contributions for the "Background" and "Discussion" sections of the manuscript. LA and JAP reviewed the manuscript and made useful suggestions for the "Discussion" and "Conclusions" sections. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Being a retrospective observational study of patients from an anonymized dataset, involving only recording data from the medical record, the Ethics Committee from Coimbra University Hospital Centre (CHUC) waived the need of informed consent according to the Declaration of Helsinki and the Council for International Organizations of Medical Sciences (CIOMS) International Ethics Guidelines.

Consent for publication

As all data was anonymized, this study does not contain any individual person's data in any form (including individual details, images, or videos) and accordingly consent for publication was waived.

Competing interests

The authors declare that they have no competing interests.

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Chapter 5 Procalcitonin kinetics after burn injury and burn surgery in septic and non-septic patients: a retrospective observational study

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RESEARCH ARTICLE Open Access

Procalcitonin kinetics after burn injury and burn surgery in septic and non-septic patients – a retrospective observational study

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Abstract

Background: Early sepsis diagnosis is crucial for the correct management of burn patients, and it clearly influences outcomes. The systemic inflammatory response triggered by burns mimics sepsis presentation and complicates early sepsis diagnosis. Biomarkers were advocated to aid the diagnosis of early sepsis. Serum procalcitonin (PCT) exhibits fair accuracy and good correlation with sepsis severity, being used in diverse clinical settings. However, few studies have evaluated perioperative changes in PCT levels in burn patients. The present study evaluated PCT kinetics during the first days after burn injury and subsequent surgical interventions to assess PCT utility in distinguishing septic from non-septic inflammatory responses.

Methods: This study was a retrospective observational study of all burn patients admitted to the Coimbra Burns Unit (Portugal) between January 2011 and December 2014 who presented with a total burn surface area ≥ 15% and who underwent subsequent surgery. PCT kinetics were investigated a) during the first five days after burn injury and b) preoperatively during the five days after surgery in three subsets of patients, including those with no preoperative and no postoperative sepsis (NN), no preoperative but postoperative sepsis (NS), and preoperative and postoperative sepsis (SS). A total of 145 patients met the selection criteria and were included in the analysis.

Results: PCT levels in the first five days after burn injury were significantly higher in patients who developed at least one sepsis episode ($n = 85$) compared with patients who did not develop sepsis ($n = 60$). PCT values > 1.00 ng/mL were clearly associated with sepsis. Study participants ($n = 145$) underwent a total of 283 surgical interventions. Their distribution by preoperative/postoperative sepsis status was 142 (50.2%) in NN; 62 (21.9%) in NS; and 79 (27.9%) in SS. PCT values exhibited a parallel course in the three groups that peaked on the second postoperative day and returned to preoperative levels on the third day or later. The lowest PCT values were found in NN, and the highest values were observed in SS; the NS values were intermediate.

Conclusions: PCT kinetics coupled with a clinical examination may be helpful for sepsis diagnosis during the first days after burn injury and burn surgery.

Keywords: Burns, Sepsis, Procalcitonin, Surgery

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Background

An early diagnosis of sepsis is of the utmost importance for the correct management of burn patients because it has a marked impact on treatment outcomes and survival [1]. Sepsis can lead to multiple organ dysfunction syndrome (MODS), which is the cause of most deaths in burn units [2]. Therefore, a prompt sepsis diagnosis and the immediate initiation of antimicrobial therapy are needed to reduce morbidity and mortality. However, the unnecessary administration of antimicrobials is often associated with adverse effects, increased costs and the emergence and spread of antimicrobial resistance.

It is clinically difficult to identify patients who are developing sepsis because the overwhelming systemic inflammatory response triggered by burn trauma mimics the signs and symptoms of sepsis [3]. A definitive diagnosis of sepsis requires microbiological cultures, but the results are not available for 24 to 48 h, and false negative results are found in 20–30% of cases. Therefore, the development of complementary tools for sepsis diagnosis, such as the use of biomarkers, is necessary [4].

Biomarkers and their kinetics may aid the clinical examination in the differentiation of infectious from non-infectious inflammatory responses [5, 6]. Numerous sepsis biomarkers are described in the literature [7], and procalcitonin (PCT) is one of the most studied biomarkers. PCT exhibits the best discriminative power of all of the biomarkers that are available at most hospital facilities [8, 9]. Thyroid C cells primarily secrete PCT in healthy subjects, and it is barely detected in blood (< 0.01 ng/mL). Many other cell types (liver, kidney, adipocytes, etc.) secrete PCT in response to direct or indirect infectious stimuli during septic episodes, and it is massively released into the bloodstream at concentrations that reach 1000 times its normal values [10]. Increased PCT is noticeable 2–4 h after sepsis onset and peaks at 24–48 h. PCT levels decrease by 50% every 1–1.5 days (half-life) when the infectious process is controlled [11]. PCT levels are highly correlated with bloodstream infections [12], and a recent meta-analysis demonstrated that elevated PCT levels and PCT non-clearance were related with an increased risk of sepsis and a higher mortality rate [13]. PCT is accurate for sepsis diagnosis, and its kinetics exhibit good correlation with sepsis severity [14]. Therefore, PCT is recommended in diverse clinical settings, including the exclusion of a bacterial cause in lower respiratory infections [15] as well as the diagnosis, stratification, prognosis [16, 17] and antimicrobial administration guidance in septic patients [18] and the diagnosis of postoperative infections [19, 20]. However, the utility of PCT in burn patients was questioned because of the high rate of false-positive results from the systemic inflammatory response induced by burn injury and subsequent surgical interventions [21, 22].

The present study evaluated PCT kinetics after a burn episode and the surgical intervention(s) needed for its treatment to assess its utility in the differential diagnosis between septic and non-septic inflammatory responses.

Methods

Study plan

This retrospective observational study used clinical and laboratory data collected from the health records of all burn patients admitted to Coimbra Burns Unit (CBU), a department of Coimbra Hospital and University Centre (CHUC), a tertiary referral hospital in Portugal, between January 2011 and December 2014, who presented with a 15% or more total burn surface area (TBSA) and who underwent subsequent surgery during their hospitalization. A total of 145 patients met the selection criteria, and their data were available for analysis.

Sepsis was diagnosed according to the American Burn Association (ABA) criteria [23]: presence, in at least one of the initial five days, of a clinical suspicion of infection coupled with at least three of the following findings: temperature > 39 °C or < 36.5 °C, tachycardia > 110 beats/min, tachypnea > 25 breaths/min or minute ventilation > 12 L/min, thrombocytopenia < 100,000/mL, hyperglycaemia (untreated plasma glucose > 200 mg/dL or intravenous glucose requirement > 7 U/h over 24 h), and enteral feeding intolerance (abdominal distension or gastric residuals more than two times feeding rate or diarrhoea > 2500 mL).

Serum PCT concentrations were measured using TRACE© (time-resolved amplified cryptate emission) technology (Kryptor© PCT; Brahms© AG; Hennigsdorf, Germany).

PCT kinetics were evaluated in the first five days after burn injury in the entire study population, preoperatively and during the five days after surgery in three subsets of patients: no preoperative and no postoperative sepsis (NN), no preoperative but postoperative sepsis (NS), and preoperative and postoperative sepsis (SS).

Statistical analysis

The maximum value of PCT on each day of the study was used for statistical analyses.

Qualitative variables (e.g., gender and mortality) are described as counts, and quantitative variables (e.g., TBSA and ABSI - Abbreviated Burn Severity Index: see Additional file 1) are described as the means and corresponding standard deviations. The number of surgical interventions and PCT values by subgroup are described as medians and interquartile ranges (IQR). Comparisons between sepsis and no sepsis groups were performed using the Mann-Whitney test for quantitative variables and the Fisher's exact test for qualitative variables. Time comparisons of PCT levels were performed using Friedman's test.

Receiver operating characteristic (ROC) curves and comparative analysis of the area under the curve (AUC)

Table 1 Study population

Characteristics	No Sepsis	Sepsis[#]	*p*-value
Number of patients	85	60	
Gender (male/female)	45/40	39/21	0.115
Age (years)[$]	56.49 (±18.15)	58.43(±21.89)	0.517
ABSI score[a]	7.69 (±2.82)	9.17 (±2.20)	0.000*
TBSA (%)[$]	29.97 (±19.94)	34.6 (±17.26)	0.000*
Mortality (No/Yes)	84/1	24/36	0.004*

*p-values < 0.05
[$]Values are Median (Q1-Q3)
[#]At least one day with sepsis in the first five days after burn episode
[a]Description in Annex I

were performed to evaluate the discriminatory power of PCT levels on consecutive days.

Statistical analysis was performed using SPSS© 23.0 IBM© for Windows©, and a p-value ≤0.05 was considered significant.

Results

Table 1 presents the primary demographic and baseline characteristics of the study population, which consisted of 84 males and 61 females. The sepsis ($n = 85$) and non-sepsis ($n = 60$) groups showed no significant differences in terms of gender or age, but they were significantly different in terms of ABSI score, TBSA and mortality.

The analysis of PCT levels during the first five days after the burn episode showed a statistically significant difference between the group of patients who developed at least one sepsis episode during that time and the group of patients who did not develop sepsis (Fig. 1). PCT values over 1.00 ng/mL were clearly associated with septic processes ($p < 0.001$, Mann-Whiney U test, Table 2). ROC curves and the AUC were performed to evaluate the discriminatory power of PCT over consecutive days. These results demonstrated that the discriminatory power of PCT levels increased over time (Table 3 and Fig. 2).

All patients ($n = 145$) underwent at least one surgical intervention, with a cumulative 283 surgical interventions. Each patient was subjected to a median of three interventions, with an IQR of [2.00–5.25]. All interventions were performed under general anaesthesia and were classified as clean-contaminated. The interventions consisted primarily of escharectomies, skin autografts and flaps, and digits/limb amputations to a lesser extent.

To assess the influence of surgical trauma on PCT concentrations, PCT evolution from the day before the operation (D0) until the fifth postoperative day (D5) was analysed. Differences in the time evolution of PCT between the sepsis and non-sepsis groups were statistically significant (Table 4), and the discriminatory power increased over time as shown by the ROC curve analysis (Table 5 and Fig. 3).

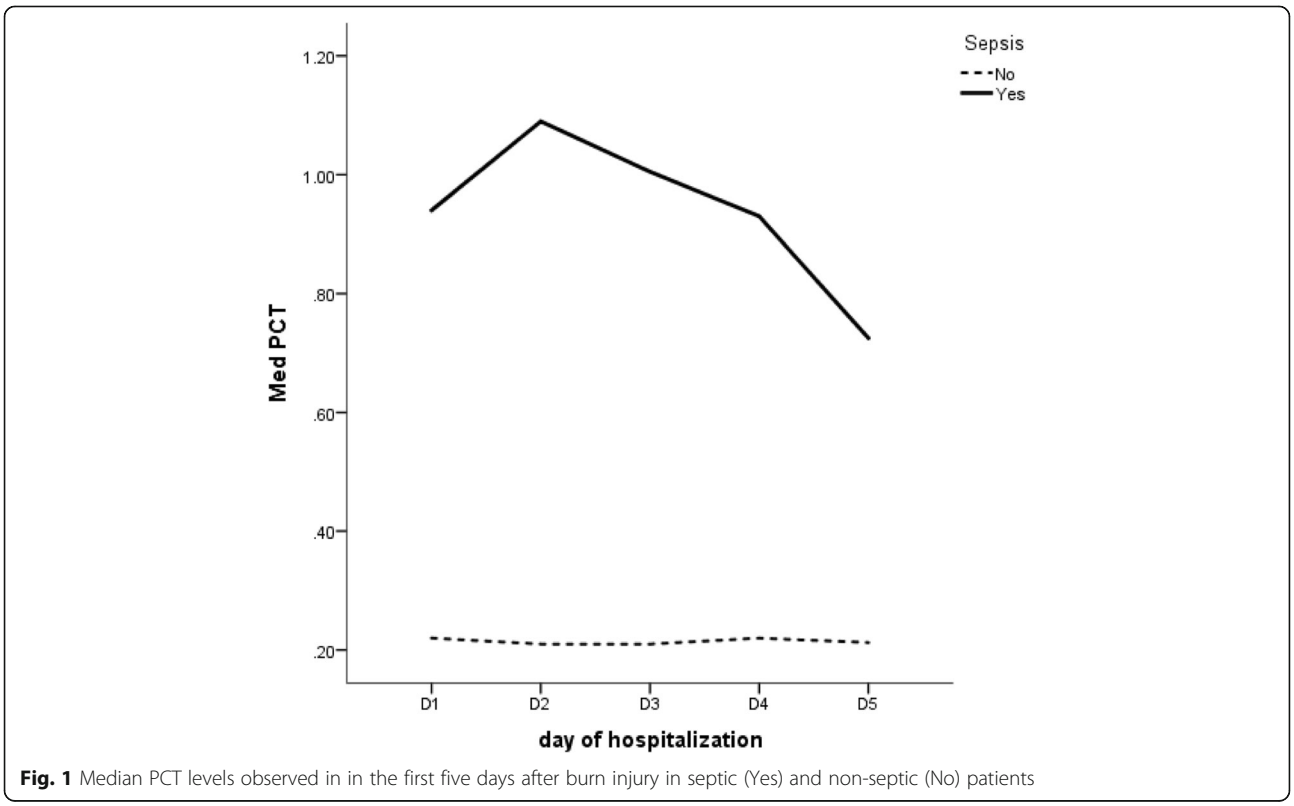

Fig. 1 Median PCT levels observed in in the first five days after burn injury in septic (Yes) and non-septic (No) patients

Table 2 Statistical analysis of PCT kinetics in the first five days after burn injury in septic and non-septic patients

Sepsis	Statistic	D1	D2	D3	D4	D5	*p*-value*
No	n	64	70	72	69	62	
	Median [IQR]	0.215 [0.090–0.578]	0.205 [0.09–0.723]	0.210 [0.08–0.668]	0.215 [0.102–0.695]	0.213 [0.118–0.618]	0.557
	n	50	53	52	53	52	
Yes	Median [IQR]	1.085 [0.188–5.440]	1.650 [0.235–4.010]	1.130 [0.335–2.920]	1.060 [0.355–2.927]	0.725 [0.340–2.105]	0.288
	p-value**	0.001	0.000	0.000	0.000	0.000	

*Friedman test
**Mann-Whiney U test

Regarding the preoperative/postoperative sepsis status, median values had a parallel course in the three groups (Fig. 4). Values peaked in the second postoperative day and returned to preoperative levels on the third day or later. The lowest values were found in the NN group, which included 142 surgical interventions in patients without preoperative sepsis and who did not develop postoperative sepsis through D5 (50.2%). The highest values were observed in the SS group, which included 62 surgical interventions in patients with pre- and postoperative sepsis (21.9%). Group NS exhibited PCT values roughly in the middle range between the other two groups and included 79 surgical interventions in patients who did not exhibit septic processes preoperatively but developed sepsis on at least one of the five days after surgery (27.9%). The kinetics of the PCT levels within each group (Table 3) were significantly different between days after surgery in the absence (NN) or presence of sepsis (NS and SS).

Discussion

The present study included a sample of 145 burn patients from the CBU, and PCT levels were significantly different between septic and non-septic patients during the first five days after burn injury. The results indicate that PCT values evolved in parallel with sepsis development and the antimicrobial therapy effect. In this important population, PCT consistently showed good potential to discriminate between septic and non-septic patients, particularly when frequent PCT assays were performed and when its kinetics were dynamically assessed.

Table 3 ROC curves for the discriminatory power of PCT levels between septic and non-septic patients in the first five days after burn injury

Area Under the Curve (AUROC)			
Day	Area	Asymptotic 95% Confidence Interval	
		Lower Bound	Upper Bound
D1	0.646	0.532	0.759
D2	0.704	0.598	0.810
D3	0.746	0.647	0.845
D4	0.752	0.654	0.850
D5	0.741	0.641	0.841

To evaluate PCT performance after surgical interventions and to investigate whether surgical trauma alone could reduce the accuracy of the diagnosis of postoperative sepsis, this study included a substantial and diversified number of interventions performed in the three subsets of patients who were organized according to the existence or absence of preoperative sepsis and the development or worsening of sepsis after surgery. PCT levels increased modestly and rapidly returned to basal levels after the second postoperative day in patients with no preoperative or postoperative sepsis episodes. Patients with increased preoperative PCT values that corresponded to preoperative sepsis exhibited PCT kinetics with a higher peak on the second postoperative day, which was presumably related to the additive increment of PCT of surgical trauma. PCT values returned to the initial values when antimicrobial therapy was administered. PCT levels in patients who only developed sepsis after surgery exhibited a parallel evolution to the already septic patients but generally with lower absolute values. Therefore, PCT is useful for sepsis diagnosis in cases of surgical intervention when preoperative PCT values are known because PCT kinetics follow the same pattern of evolution in cases of sepsis as in other critical patients.

The search for sepsis biomarkers is an exciting and never-ending story [24, 25]. Diverse approaches were used to identify more precise, practical, quicker, safer and cheaper chemicals or physical changes that may indicate the urgent need and adequacy of antimicrobial therapy or its redundancy to reduce adverse events, microbial resistance and financial costs. Current research is more focused on molecular (PCR, MALDI-TOF) and/or system-based (genomics, transcriptomics, proteonomics, metabolomics) methods for sepsis diagnosis [26–29], but these techniques are not fully developed, practical or widely available.

An ideal biomarker is not developed, and the use of PCT as an early distinction between actual septic patients and patients with merely systemic inflammatory signals and symptoms during the first days after hospital admission has been largely discussed in the medical literature in the last two decades [30–35]. PCT is a useful but not ideal

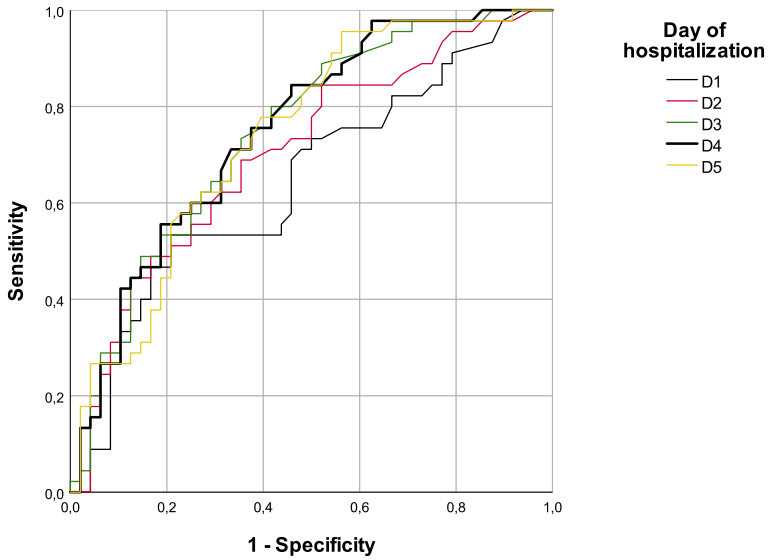

Fig. 2 ROC Curves for the discriminatory power of PCT levels between septic and non-septic patients in the first five days after burn injury

biomarker, particularly due to its negative predictive power [36], which led to its inclusion in algorithms for sepsis management [37, 38]. The use of serial measurements instead of a single observation reinforces the predictive power of PCT and reduces the risks of false negatives and false positives [39–43]. The same considerations are valid for the use of PCT in the investigation of suspected postoperative sepsis [44], which is currently performed after many types of surgical procedures [45–49].

PCT accuracy in burn patients is controversial [21, 22, 50, 51]. Burn patients are generally excluded from sepsis studies and clinical trials based on the simplistic assumption that PCT levels are always elevated in burn patients as a result of the non-septic inflammatory systemic response related to burn trauma. However, several studies consistently demonstrated different PCT kinetics in burn patients based on the presence or absence of systemic infection [52–55]. Three recent meta-analysis also validated the use of PCT for sepsis diagnosis in these patients [56–58]. PCT evolution is predictable in both cases, and it provides a reliable means to identify septic processes, which was first referred to by von Heimburg et al. in 1998

Table 4 Statistical analysis of PCT kinetics from preoperative day (D0) till the fifth postoperative day (D5) for NN, NS and SS groups

Sepsis	Statistic	D0	D1	D2	D3	D4	D5	*p*-value*
NN	n	212	208	198	186	163	149	
	Median [IQR]	0.190 [0.110–0.560]	0.200 [0.101–0.615]	0.280 [0.120–0.758]	0.223 [0.118–0.553]	0.195 [0.110–0.430]	0.180 [0.100–0.360]	0.000
NS	n	104	103	102	100	87	80	
	Median [IQR]	0.405 [0.219–0.935]	0.510 [0.240–1.360]	0.640 [0.313–1.590]	0.625 [0.283–1.438]	0.540 [0.260–1.970]	0.515 [0.273–2.045]	0.000
SS	n	74	74	74	69	65	62	
	Median [IQR]	0.653 [0.233–2.193]	0.790 [0.288–2.518]	1.115 [0.413–2.990]	0.880 [0.380–3.115]	0.710 [0.300–1.950]	0.580 [0.248–1.520]	0.000
	p-value**	0.000	0.000	0.000	0.000	0.000	0.000	
	Multiple comparison (*p*-value***)							
(NN,NS)		0.000	0.000	0.000	0.000	0.000	0.000	
(NN,SS)		0.000	0.000	0.000	0.000	0.000	0.000	
(NS,SS)		0.339	0.225	0.135	0.294	1	1	

*Friedman test
**Kruskal Wallis test
***Mann-Whiney U test with Bonferroni correction

Table 5 ROC curves for the discriminatory power of PCT levels between septic and non-septic patients preoperatively and in the first five days after burn surgery

Area Under the Curve (AUROC)			
Day	Area	Asymptotic 95% Confidence Interval	
		Lower Bound	Upper Bound
D0	0.662	0.591	0.733
D1	0.701	0.633	0.770
D2	0.717	0.649	0.784
D3	0.752	0.686	0.815
D4	0.760	0.696	0.824
D5	0.771	0.708	0.834

[59]. The immediate inflammatory burst elevates PCT levels after burn injury, independent of infection, and grossly correlates with TBSA, but it rarely surpasses 2.0 ng/mL [60, 61]. The maximum PCT value is reached within 24–48 h in the absence of sepsis and returns to normal values (1.0–1.5 ng/mL or less) by the end of the third day. PCT levels continue increasing in the presence of sepsis and rapidly reach values greater than 5–100 ng/mL. PCT levels only diminish with antimicrobial therapy or terminal immunosuppression, as observed in other forms of severe trauma [62]. Lavrentieva et al. analysed a sample of 145 burn patients and found increased PCT levels during the first 24 h after a burn episode, which subsided in non-septic patients and continued increasing in septic patients. These authors demonstrated an inverse relationship of PCT level tendency with antimicrobial therapy efficacy. They proposed a cut-off of 1.5 ng/mL to distinguish between septic and non-septic patients [63]. Egea-Guerrero et al. [64] and Kim et al. [65] found the same PCT kinetics and approximate cutoffs.

PCT exhibited a similar kinetics pattern after surgical intervention [66], but preoperative PCT levels must be known to use these levels to discriminate between the postoperative physiological inflammatory response and postoperative sepsis. Preoperative PCT levels are related to the presence or absence of an ongoing sepsis process and possible ongoing antimicrobial therapy, which naturally influence baseline values [67]. To the best of our knowledge, the present study is the first study to specifically address PCT kinetics after surgical procedures in burn patients and demonstrate that this biomarker maintains its performance in this particular set of patients, even in the presence of preoperative sepsis.

PCT levels coupled with rigorous clinical monitoring and blood cultures as the diagnostic cornerstone [68] may help confirm or exclude sepsis in patients during the acute phase after burn trauma and ascertain the presence of postoperative sepsis in burn patients. Neither immunodepression [69] nor corticotherapy [70] affected the diagnostic performance of PCT, as opposed to other biomarkers, and PCT also distinguishes contamination from actual bloodstream infection [71]. The use of PCT dosing may inclusively reduce healthcare costs and avoid the superfluous use of antimicrobials and consequent increments on microbial resistance [72, 73].

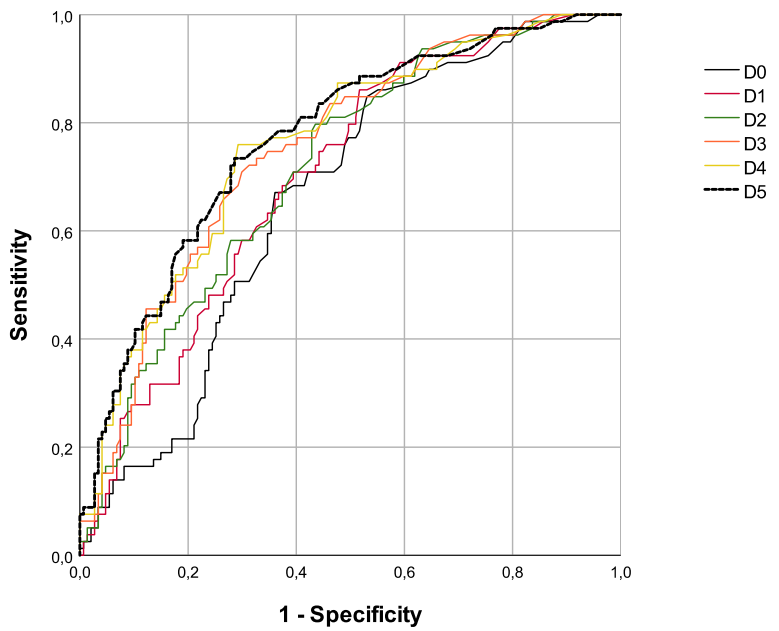

Fig. 3 ROC Curves for the discriminatory power of PCT levels between septic and non-septic patients preoperatively and in the first five days after burn surgery

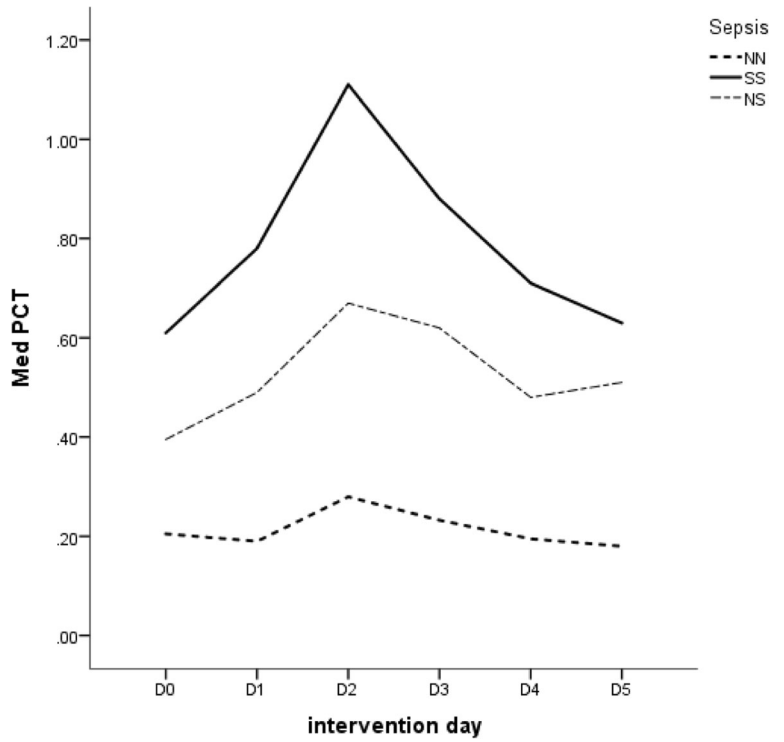

Fig. 4 Median PCT levels observed from preoperative day (D0) till the fifth postoperative day (D5) for NN, NS and SS groups

The present study also presents some limitations. First, it was a single-centre, retrospective observational study, and the results require confirmation using prospective multicentre trials. Second, the precise influence of antimicrobial therapy in septic patients could not be evaluated because of ethical considerations that naturally prevent antimicrobial denial in face of septic episodes. Third, subgroup analyses according to the total burned surface area (TBSA) and the severity of patients' attainment, for instance, using the Abbreviated Burn Severity Index (ABSI), was not performed. However, the use of defined and internationally accepted criteria for the clinical suspicion of burn sepsis, the homogeneity of therapeutic procedures, and the use of a standard methodology for the collection, recording and statistical analysis of the data are clearly strengths of the present study.

Conclusion

The present study was performed in 145 burn patients who underwent a high and diversified number of surgical interventions. The results allow us to conclude that 1) PCT kinetics may aid in the differential diagnosis between true sepsis and the normal inflammatory response to burn trauma in the first days after burn injury; and 2) PCT kinetics may be used to identify postoperative sepsis in burn patients who undergo surgical interventions during their stay in burn units.

Prospective multicentre studies in adult and paediatric burn patients are needed to confirm these findings and compare PCT and other biomarkers in these contexts.

Additional file

Additional file 1: Abbreviated Burn Severity Index. (DOCX 17 kb)

Abbreviations

ABA: American Burn Association; ABSI: Abbreviated Burn Severity Index; AUC: Area Under the Curve; CBU: Coimbra Burn Unit; CHUC: Coimbra University Hospital Centre (Centro Hospitalar e Universitário de Coimbra); CIDMA: Center for Research and Development in Mathematics and Applications, Department of Mathematics, University of Aveiro; CIOMS: Council for International Organizations of Medical Sciences; iBiMED: Institute for Biomedicine, Department of Mathematics, University of Aveiro; IBM: International Business Machines; IQR: Interquartile Range; MALDI-TOF: Matrix-Assisted Laser Desorption Ionization – Time of Flight; MedinUP: Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto; MODS: Multiple Organ Dysfunction Syndrome; PCR: Polymerase Chain Reaction; PCT: Procalcitonin; ROC: Receiver Operating Characteristic; SACS: Autonomous Section of Health Sciences (Secção Autónoma de Ciências da Saúde), University of Aveiro; SPSS: Statistical Package for the Social Sciences; TBSA: Total Burn Surface Area; TRACE: Time-Resolved Amplified Cryptate Emission

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Availability of data and materials
The data that support the findings of this study are available from the datasets of the Clinical Pathology Department and the Informatics Department of Coimbra University Hospital Centre, but restrictions apply to the availability of these data, which were used under license for the current study and are not publicly available. However, data are available from the authors upon reasonable request and after permission of the Ethics Committee from Coimbra University Hospital Centre.

Authors' contributions
All authors read the manuscript and agreed to its content. All authors are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with ICMJE criteria and provided their consent for publication. LC, VA, MM and IT designed the study, interpreted data and drafted the manuscript. VA was responsible for most of the statistical analyses. RM, MV and CC were responsible for data acquisition and literature search and offered suggestions for citation integration in the manuscript. CC also made substantial intellectual contributions for the Introduction and Discussion sections of the manuscript. LA and JAP reviewed the manuscript and provided useful suggestions for the Discussion and Conclusion sections.

Ethics approval and consent to participate
This study has got the formal approval from the Ethics Committee of Coimbra University Hospital Centre (CHUC) to its realization. The Ethics Committee of Coimbra University Hospital Centre (CHUC) also waived the need for informed consent according to Declaration of Helsinki and CIOMS International Ethics Guidelines because this study was a retrospective observational study of patients from an anonymized dataset that only involved recording data from medical records.

Consent for publication
All data was anonymized, and this study does not contain any individual person's data in any form (including individual details, images or videos). Therefore, consent for publication was waived.

Competing interests
On behalf of all authors, the corresponding author states that they have have no competing interests.

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Chapter 6 Evaluation of procalcitonin accuracy for the distinction between Gram-negative and Gram-positive bacterial sepsis in burn patients

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Evaluation of Procalcitonin Accuracy for the Distinction Between Gram-Negative and Gram-Positive Bacterial Sepsis in Burn Patients

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Sepsis is the main cause of death in burns. Early institution of antimicrobial therapy is crucial to optimize outcomes but superfluous therapy increases adverse events, microbial resistance, and costs. Blood cultures are the gold standard for diagnosis but can take 48 to 72 hours. Biomarkers are used to help sepsis diagnosis and distinction between Gram-negative and Gram-positive bacterial cause. The aim of this work is to evaluate procalcitonin (PCT) accuracy for this distinction in burn patients. Retrospective observational study of adult septic burn patients with ≥15% total burn surface area admitted from January 2011 to December 2014 at a Burn Unit in Portugal. A statistical analysis was done, evaluating the correlation between PCT levels on the day of the first positive blood culture and microbiological data for Gram-negative and Grand-positive bacteria. Patients with mixed bacterial and/or fungal blood cultures were excluded. Data were summarized by quartiles statistics. Blood cultures were positive in 189 patients: 75 (39.7%) showed growth for Gram-negative and 114 (60.3%) for Gram-positive bacteria. Patients with Gram-negative bacteria have significantly higher PCT levels. Receiver operating characteristic curve analysis showed accuracy for Gram-negative discrimination with area under the curve = 0.687. Most elevated levels were related to nonfermentative Gram-negative bacteria and by *Klebsiella pneumoniae* and other Enterobacteriaceae. PCT levels were significantly higher in burn patients with Gram-negative sepsis comparing to patients with Gram-positive sepsis and controls. The determination of PCT levels may help the choice of empirical antimicrobial therapy while microbiological culture results are not available, despite not fully ensuring the desirable degree of precision.

An early and adequate antimicrobial therapy is the main step for the management of septic patients.[1] Unfortunately, differential diagnosis between sepsis and the systemic inflammatory response triggered by trauma is difficult, particularly in burn patients where the usual clinical signs of sepsis are frequently present even in the absence of microbial infection.[2] For instance, burn injuries leading to upregulation of the hypothalamic thermal center, physiologic release of catecholamines and cytokines, shift of fluids and the consequent cardiovascular changes, can produce hyperthermia, tachycardia, hypotension, etc., that are transitory and do not reflect any microbial invasion but just a tentative of adjustment of human body systems to the changes in the homeostatic equilibrium.

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Authors' Contributions. All authors have read the manuscript and agreed to its content, are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with ICMJE criteria and gave their consent for publication. L.C., V.A., C.C., and M.C. designed the study, interpreted data, and draft the manuscript. V.A. was responsible for most of statistical analysis. R.M., M.V., and J.G.F. were responsible for data acquisition, search of literature, and made suggestions for its integration along the manuscript. C.C. made also substantial intellectual contributions for the Introduction and Discussion sections of the manuscript. L.A. and J.A.P. review the manuscript, and made useful suggestions for Discussion and Conclusions sections.

Ethics Approval and Consent to Participate. Being a retrospective observational study of patients from an anonymized dataset, involving only recording data from the medical record, the Ethics Committee from Coimbra University Hospital Centre (CHUC), waived the need of informed consent according to Declaration of Helsinki and CIOMS International Ethics Guidelines.

Consent for Publication. As all data was anonymized, this study does not contain any individual person's data in any form (including individual details, images, or videos) and accordingly consent for publication was waived.

Availability of Data and Material. The data that support the findings of this study are available from the datasets of the Informatics Department of Coimbra University Hospital Centre but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors, upon reasonable request and after permission of the Ethics Committee from Coimbra University Hospital Centre.

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The gold standard for sepsis diagnosis still relies on the microbiological growth in blood cultures,[3] which can take as long as 48 to 72 hours, according to different facilities,[4] and the antimicrobial sensibility tests may be available even later. On the other hand, the adequacy of antimicrobial therapy is obviously related with the appropriateness of the chosen drugs, that is, the selection of the most efficacious drug against the causative microorganism. In practical terms, physicians have to assess the presence of sepsis in a complex clinical setting, with great chance of misdiagnosis (false positive or false negative), and in most cases to wait 2 days to confirm their suspicion, having (or not) prescribed an antimicrobial therapy that may be inefficacious against the causative bug, allowing the septic process to progress and increasing the likelihood of a fatal outcome. Moreover, a superfluous or an inappropriate antimicrobial therapy presents risks of adverse events for the patient and stimulates the development of microbial resistance, besides increasing costs.[5] In conclusion as described in a seminal work by Kumar et al,[6] it is of outstanding importance the prompt institution of an effective antimicrobial therapy, avoiding the serious risks present when this is not timely done.

In the last decade, biomarkers have been employed to help sepsis diagnosis and antimicrobial prescription and stopping. Together with infection control measures and antimicrobial therapy protocols, the use of biomarkers constitutes the backbone of most antimicrobial stewardship programs.[7] From a multitude of clinical and biochemical biomarkers described in literature, procalcitonin (PCT) became one of the most employed due to 1) its relatively good accuracy for the diagnosis of septic and nonseptic patients since the first hours of microbial invasion, helping the decision to start or postpone antimicrobial therapy, particularly if used in a dynamic approach; 2) correlation between PCT levels and sepsis severity, and 3) its rapid fall when infection is controlled.[8] Furthermore, significant differences in PCT levels have been found according to the causative pathogens, namely between Gram-negative and Gram-positive bacteria, which facilitates the choice of the drugs to be empirically used meanwhile blood culture results and sensibility tests are not available.

Even being controversial for some authors, determination of PCT serum levels has been consistently advocated for the diagnosis, prognosis, and antimicrobial stewardship in burn patients. Taking into account the different therapeutic approach to different pathogens, it is worthwhile to evaluate the discriminative potential of PCT to set the more appropriate empirical therapy. The aim of this work is to size up PCT performance for the differential diagnosis between sepsis by Gram-negative and Gram-positive bacteria in a large sample of burn patients.

MATERIALS AND METHODS

Patient Informed Consent

Considering that this was an observational study using anonymized retrospective data, the Independent Ethics Committee (Comissão de Ética para a Saúde, Coimbra Hospital University Center—CHUC, Coimbra, Portugal) waived the need of informed consent.

Study Plan

Data for this retrospective observational study was collected from the clinical files and laboratory electronic records of consecutive burn patients with 15% or more of total burn surface area, admitted from January 2011 to December 2014 at Coimbra Burn Unit (CBU), a department of CHUC. All the patients had positive blood cultures and clinical diagnosis of sepsis, following the American Burn Association criteria[9]: suspicion of infection coupled with the presence of three or more of the following parameters: temperature >39°C or <36.5°C; tachycardia >110 beats/min; tachypnea >25 breaths/ min or ventilation >12 l/min; thrombocytopenia <100,000/ ml; hyperglycemia (untreated plasma glucose > 200 mg/dl or intravenous glucose requirement > 7 U/h over 24 hours; enteral feeding intolerance: abdominal distension or gastric residuals more than two times feeding rate or diarrhea >2500 ml.

Blood cultures were obtained in a standardized way. Three samples were collected by sterile venepuncture in septic patients. Except when immediate antimicrobial therapy has to be initiated due to sound clinical or laboratorial sepsis suspicion, the collects were done in the morning (7–8 am). This collect was repeated every 2 days until clinical resolution and PCT normalization.

Using sample patients who never developed sepsis during its stay at CBU as controls, a statistical analysis was done to evaluate possible correlation of PCT levels on the day of the collection of the first positive blood culture with microbiological data, according to two groups of microorganisms: Gram-negative and Grand-positive bacteria. To avoid potential bias and simplify the analysis, patients with positive mixed bacterial and/or fungal blood cultures were excluded from the study. When a patient had more different microorganisms present in the blood cultures at different timepoints, only PCT levels of the first identification were subjected to analysis. If a patient had more than a PCT measurement on the day of collection, the highest value was used for the analysis. PCT was measured with TRACE© (time-resolved amplified cryptate emission) technology (Kryptor© PCT; Brahms© AG; Hennigsdorf, Germany).

Statistical Analysis

Data were summarized by quartiles statistics. The quantitative variables under study showed a non-Gaussian distribution and thus a nonparametric approach (Kruskall–Wallis and Mann–Whitney tests) was used to compare quantitative variables. Qualitative variables were compared with the Pearson chi-square test. For pairwise comparisons, the Bonferroni-correction was applied.

Receiver operating characteristic (ROC) curves, in particular the area under the curve (AUC), were performed to evaluate PCT ability in Gram-negative and Gram-positive discrimination. Sensitivity, specificity, positive and negative predictive values were calculated for some cutoff values including the best cutoff defined by the maximum value of Youden index (J = sensitivity + specificity − 1).

Statistical analysis was performed with SPSS© 25.0 IBM© for Windows©. A p value of less than .05 was set as the level of significance and the confidence intervals are reported with 95% confidence level.

RESULTS

The sample under analysis was composed 438 burn patients. Among these patients, 249 (56.8%) did not fulfill American Burn Association sepsis criteria neither had any growth in their blood cultures during their stay at CBU, being deemed to serve as controls. Blood cultures were positive in 189 (43.2%) patients; among from these, 75 patients (39.7%) showed growth for Gram-negative bacteria and 114 (60.3%) showed growth for Gram-positive bacteria (Table 1). The median age was 62 years for controls, 66 years for patients with sepsis by Gram-negative bacteria and 69 years for patients with sepsis by Gram-positive bacteria; the difference among groups did not reach statistical significance. The same was true for gender distribution, which showed a preponderance of the masculine sex: control patients included 152 males (61%) and 97 females (49%); the Gram-negative group was composed by 41 males (55%) and 34 females (45%) meanwhile the Gram-positive group gathered 70 males (61%) and 44 females (39%; Table 1).

On the day of the first identification of microbiological growth in blood cultures, PCT levels were significantly higher in patients with Gram-negative bacteria comparing to controls and patients with Gram-positive bacteria; the differences between controls and Gram-positive infected patients did not reach statistical significance (Table 2).

Figure 1 depicts box-plots for PCT levels in the first day of microbiological identification, clearly showing higher values for patients in the Gram-negative group in relation to control group and to Gram-positive group while the difference between these later groups is not evident.

The maximum value of the Youden index was 0.31, for a cutoff = 0.57 ng/ml. This cutoff reached a sensibility of 63% and a specificity of 68%; the corresponding positive predictive value was set in 57% and the corresponding negative predictive value achieved 74% (Table 3). This was the optimum PCT cutoff, corresponding to the maximum point of the ROC curve: higher ones were associated with lesser sensitivity and lower ones led to loss of specificity.

ROC curve is presented in Figure 2. The AUC showed a significant accuracy for Gram-negative discrimination from Gram-positive: AUC = 0.687, with 95% confidence interval = 0.609–0.765.

Subgroup analysis was performed including the most frequent Gram-negative and Gram-positive microorganisms responsible for sepsis in this sample of patients. In the Gram-negative group, the mostly frequently isolated agent was *Pseudomonas aeruginosa*, as it would be expected according to its great prevalence in many burn units, followed by *Acinetobacter* spp. and other nonfermentative bacteria, including *Burkholderia cepacia* and *Stenotrophomonas maltophilia*. The Enterobacteriaceae were also very common, namely *Escherichia coli*, *Enterobacter* spp., *Klebsiella pneumonia*, *Serratia marcescens*, *Proteus mirabilis*, etc. From the Gram-positive group, *Staphylococcus epidermidis*, *Staphylococcus hominis* and other coagulase-negative species of Staphylococci, some of them without more specific identification furnished by the laboratory, were the most isolated from the blood samples. As it happened with *Bacillus* spp. and *Corynebacterium* spp., most of times the coagulase-negative species of Staphylococci species were suggested to be probable contaminants in the microbiological results and sensibility tests. Group D Enterococci (namely *Enterococcus faecalis* and *Enterococcus faecium*) and *Staphylococcus aureus* were also very frequently isolated and there were also isolations of *Streptococcus* spp. Table 4 displays the list of the most common microorganisms and the corresponding values of PCT levels on the first day of microbiological identification. The full list can be found in Supplementary Annex I.

Despite the presence of several outliers, it was found that PCT levels in the Gram-negative group were in general significantly higher comparing to controls, what did not happen in the Gram-positive group, with the exception of patients with sepsis due to *Streptococcus* spp. (Figure 3). With the exception of those with sepsis due to this Gram-positive species, which isolation is rare ate CBU, in almost all patients with PCT concentrations above 3.00 ng/ml on the day of collection of the first positive blood culture, the causative microorganism was a Gram-negative agent.

In the first case, the statistical difference was more pronounced for glucose nonfermenting bacilli (particularly *Acinetobacter*, *Pseudomonas*, and *Burkholderia* spp.) and for *E. coli* and *K. pneumoniae*, glucose fermenting rods from the Enterobacteriaceae family. Among patients with sepsis due to Gram-positive cocci, PCT levels only reach statistically significant difference for *Streptococcus* spp., as referred, but there was a trend for significance for *Enterococcus* spp. and for *S. aureus* (not visible for nonaureus species).

DISCUSSION

The statistical analysis of PCT levels on the first day of microbiological identification in blood samples in this sample

Table 1. Population characteristics

		Controls	Gram-Negative Sepsis	Gram-Positive Sepsis	*P*
Number of Patients		249	75	114	—
Age (years)	Median	62.0	66.0	69.0	
	Q1–Q3	45.5–78.0	44.5–79.5	47–80.0	.392*
Sex	Males	152 (61%)	41 (55%)	70 (61%)	
	Females	97 (49%)	34 (45%)	44 (39%)	.578†
Procalcitonin (ng/ml)	Median	0.20	0.75	0.32	.000*
	Q1–Q3	0.11–0.84	0.35–4.15	0.16–0.87	

*Kruskall–Wallis test.
†Chi-square test.

Table 2. Pairwise comparisons for procalcitonin levels between sepsis groups

Comparison	*P*
Gram-negative septic patients vs controls	.000
Gram-negative septic patients vs Gram-positive septic patients	.000
Gram-positive septic patients vs controls	.153

Mann–Whitney test with Bonferroni-corrected *P*-values.

of extensively burned patients confirmed previous reports demonstrating significantly higher values in the presence of Gram-negative bacteria comparing with controls or patients with Gram-positive sepsis.[10–13] The difference was most pronounced when causative agents were glucose nonfermenting bacilli, particularly *Acinetobacter* and *Pseudomonas* spp., or Enterobacteriaceae rods, like *E. coli* or *K. pneumoniae*. On the other hand, a statistical difference in PCT levels was not found between in PCT levels of patients with sepsis caused by Gram-positive bacteria and control patients, with the exception of patients with sepsis caused by *Streptococcus* spp.

The results of this work are consistent with medical literature. Opal and Cohen[14] attributed the different characteristics of sepsis caused by Gram-negative and Gram-positive to the different constitution of their respective cell membranes, which will trigger different immunological responses and are, in most part, correlated with diverse clinical presentations and outcomes.[15] Briefly explaining, despite there is not yet a full understanding of the mechanisms involved in cytokines activation following microbial insult, it is consensual that human innate immune cells (macrophages, neutrophils, dendritic cells) have receptors, present either on the external cell membrane or inside the cytoplasm (endosomes) which are apt to recognize specific circulating molecular patterns. These pattern recognition receptors (PRRs) can be activated by molecular patterns resulting from nonmicrobial tissue damage (damage-associated molecular patterns, DAMPs) or by those exclusively corresponding to microbial pathogenic components (pathogen-associated molecular patterns, PAMPs).[16] The interaction between PRRs and PAMPs induces the release of cytokines by immune cells, initiating the septic process.

There are several types of PRRs, including Toll-like receptors (TLRs) and NOD-like receptors—mainly activated by bacteria; RIG-I-like receptors and DNA-sensing molecules—crucial for sensing of viruses; C-type lectin receptors responding to fungi and mycobacteria PAMPs; etc. The outer membrane of Gram-negative bacteria cell wall is composed mostly by lipopolysaccharide, frequently referred as endotoxine, which is its principal PAMP, being recognized by TLR4.[17] Instead of lipopolysaccharide, PAMPs of Gram-positive bacteria cell wall are basically lipoteichoic acid,[18] lipoproteins and proteoglicans, mostly sensed by TLR2.

TLR4 activation triggers a strong release of inflammatory cytokines, namely tumor-necrosis factor α, interleukin-1, and interleukin-6.[19] These cytokines will promote gene transcription leading to PCT secretion from extrathyroidal tissues, with abrupt rise of its blood levels. It was also described a direct stimulation of PCT secretion by circulating endotoxins.[20] On the other hand, TLR2 activation usually induces a relatively weaker and not always straightforward production of those cytokines, varying according to different pathogens by not well known reasons.

In 2008, Charles and colleagues analyzed the accuracy of PCT measurements to discriminate between Gram-negative and Gram-positive bacteremia at the onset of bloodstream infection, concluding that serum levels were greater in the

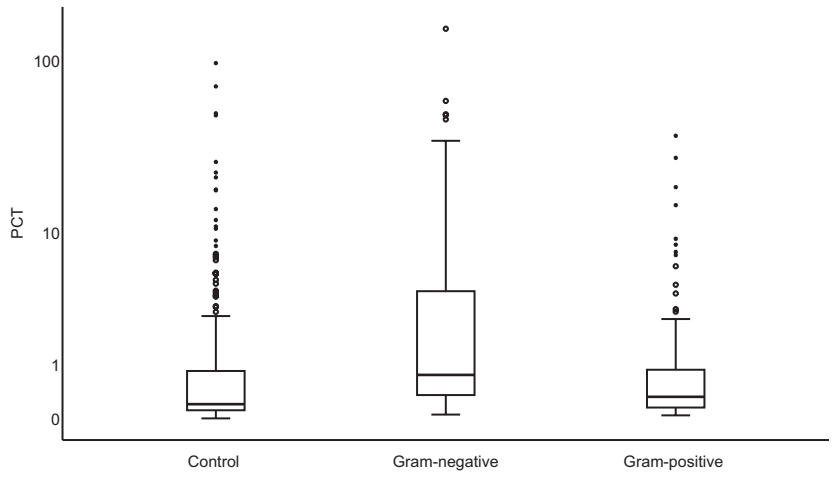

Figure 1. Box-plots for procalcitonin levels in controls (n = 249), Gram-negative (n = 75), and Gram-positive (n = 114) sepsis patients groups.

Table 3. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of procalcitonin cutoffs for the distinction between Gram-negative and Gram-positive sepsis in burn patients

Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden	Obs.
0.50	64	62	53	72	0.26	
0.57	63	68	57	74	0.31	Max. Youden
1.00	46	76	52	71	0.22	
5.00	23	92	62	68	0.15	

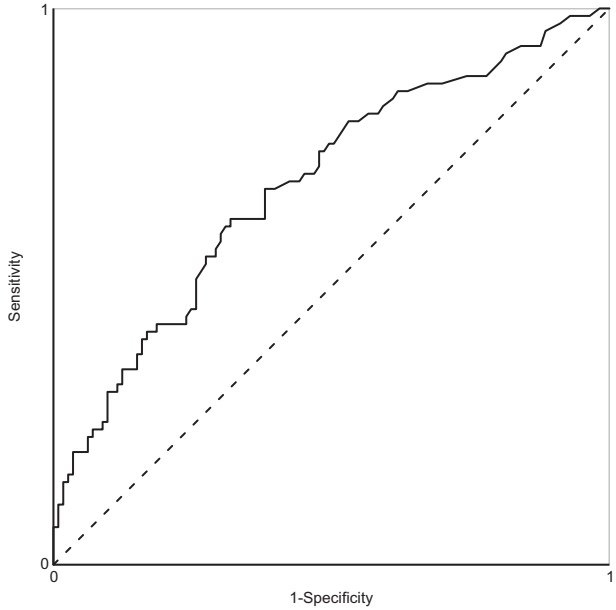

Figure 2. Receiver operating characteristic curve for procalcitonin accuracy in the distinction between Gram-negative and Gram-positive bacterial sepsis in burn patients (cutoff = 0.57 ng/ml).

first group, with an AUC of 0.79, opposing to what happened with the measurements of C-reactive protein and leucocyte counting.[21] As PCT levels determination is available sooner than Gram stain results and microbiological identification, these authors suggest this information should be taking in account when choosing empirical antibiotherapy for sepsis.

In 2012, Jeong et al,[22] showed a good performance of PCT in the distinction between patients with negative and positive blood cultures, facilitating the differentiation of true bloodstream infections from contamination. They also reported that significantly higher values were found for Gram-negative bacteremia comparing to Gram-positive or fungal ones, what did not happen with C-reactive protein values. With most patients coming from hematological ICUs, an article by Brodská et al[23] described significantly higher PCT values for patients with Gram-negative bacterial infections, comparing to Gram-positive or fungal infections, meanwhile no statistical difference was found between these latter two groups. They concluded that PCT levels could be used to help the confirmation or exclusion of Gram-negative sepsis. Nakajima et al,[24] presented similar results in 2014, speculating the possibility of using PCT levels to help antimicrobial empiric antibiotherapy.

In 2015, Oussalah et al[25] using a comprehensive electronic database, performed an observational cross-sectional study and analyzing 2699 patients with positive blood cultures, found statistically higher PCT levels in patients with Gram-negative sepsis comparing patients with Gram-positive sepsis, with most elevate values for *Escherichia* spp., *Bacteroides* spp., *Klebsiella* spp. and *Enterobacter* spp. They also pointed values under 0.75 ng/ml as very effective for exclusion of most clinical relevant pathogens, meanwhile a cutoff above 10 ng/ml practically excluded the hypothesis of sample contamination or fungal infection. In a prospective study, including 1949 adult patients with positive blood cultures, Leli et al[26] also reported significantly higher PCT levels for Gram-negative infections, more pronounced for Enterobacteriaceae bacteria, suggesting a cutoff of 3.1 ng/ml for the exclusion of these microorganisms. Guo et al[27] reached the same results in a sample of 280 septic patients and listed *Klebsiella*, *Escherichia, Acinetobacter, Enterobacter*, and *Pseudomonas* as the pathogenic species responsible for higher PCT levels. In 2016, Li et al[28] analyzing 328 septic episodes, suggested that PCT levels might be used as a surrogate marker to distinguish sepsis cases originated by Gram-negative bacteria from the ones deriving from Gram-positive bacterial or fungal invasion of bloodstream, proposing a cutoff of 2.44 ng/ml. Yan et al[29] reviewed data from 484 monomicrobial positive blood cultures of septic patients (75% collected at the ICU and 25% at the Emergency Department), reporting statistically significant differences in PCT levels, with higher values corresponding to patients

Table 4. Procalcitonin values for the most frequently isolated groups of microorganisms in blood samples of septic burn patients

Microorganism	Number	Median	Q1	Q3	*P*
Controls	249	0.20	0.11	0.84	
Glucose nonfermenting Gram-negative Bacilli					
Acinetobacter spp.	13	1.17	0.49	7.30	.002
Pseudomonas spp.	13	0.67	0.39	1.68	.005
Burkholderia cepacia	4	1.82	0.89	3.05	.045
Xanthomonas maltophilia	4	0.63	0.29	8.89	.241
Enterobacteriaceae					
Enterobacter	9	0.55	0.22	0.62	.087
Escherichia coli	5	2.96	0.75	6.90	.020
Klebsiella pneumoniae	5	1.77	0.58	22.18	.043
Serratia marscecens	5	0.75	0.48	0.89	.255
Gram-positive Cocci					
Enterococcus spp.	12	0.38	0.18	0.73	.177
Staphylococcus aureus	11	0.28	0.21	0.97	.185
Staphylococcus (except aureus)	54	0.29	0.11	0.88	.668
Streptococcus spp.	8	2.18	1.27	4.91	.003

Mann–Whitney test (comparison with control).

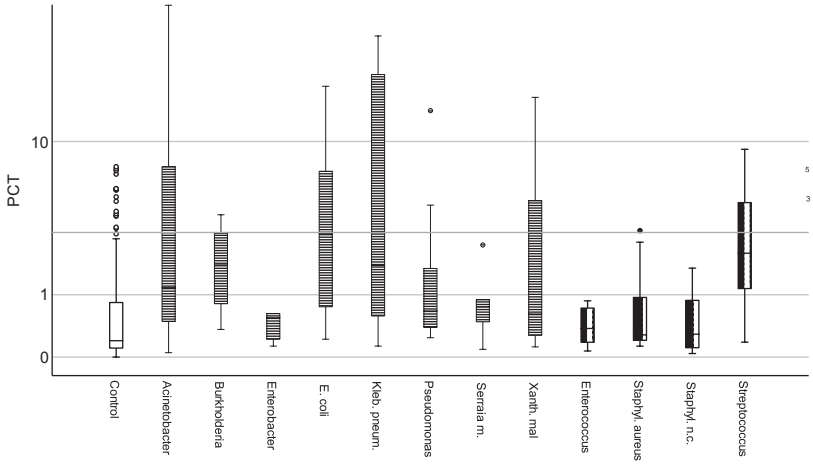

Figure 3. Box-plots for procalcitonin levels in Gram-negative and Gram-positive bacterial sepsis subgroups.

with Gram-negative infection. From the Gram-negative bacterial sepsis group, PCT levels were more pronounced for Enterobacteriaceae microorganisms (*E. coli, Kebsiella* spp., *Enterobacter* spp., and *Serratia* spp., by this order), which presented relatively higher values than glucose nonfermenting Gram-negative bacilli (*P. aeruginosa*, *Acinetobacter baumanii*, *S. maltophilia*, *B. cepacia*, etc.). From the Gram-positive bacterial sepsis group, patients infected by *Streptococcus* spp., *Enteroccoccus* spp., and *S. aureus* had the most elevated PCT concentrations. The authors defended that PCT could be useful not only to distinguish between Gram-negative and Gram-positive sepsis, but might even be employed to identify diverse species inside each of these groups of microorganisms.

In a work from 2018, Thomas-Rüddel et al performed a secondary analysis of a prospectively collected dataset, including a very large sample with 4858 septic patients from 40 hospitals. Their results were very similar to the present study, showing distinctly higher values for PCT concentrations in patients with Gram-negative bacteremia than in patients with sepsis resulting from Gram-positive or fungal systemic invasion.[30] Indeed, the AUC for the discrimination of Gram-negative sepsis from Gram-positive was identical, is spite substantially diverse cutoffs. Subgroups of pathogens with the most elevated values were also very close, with *Streptococcus* spp.; *E. coli, Proteus* spp., *K. pneumoniae* and other Enterobacteriaceae on the top. The authors referred, however, a large overlap of PCT levels and speculate that higher values may be more related with higher bacterial load and potentially with intrinsic characteristics of pathogens groups, considering the discriminatory power too low to guide therapeutic decisions.

Burn patients have a risk of infection superior to the average critical care patient and sepsis diagnosis is more difficult[31] due to the intense inflammatory systemic response unleashed by the burn insult *per se*. In these patients, PCT measurements, particularly using a kinetic approach, have being increasingly advocated by many authors to help the differentiation between pure inflammatory reaction and microbial infection[32–36] and for antimicrobial stewardship.[37] However, this strategy is still not fully accepted[38–42] in spite of systematic reviews and meta-analysis suggesting its validity.[43–45] In 2012, a study of Lavrentieva et al,[46] including 86 burn patients, was presumably the first work reporting statistically significant differences of PCT levels between burn patients with Gram-negative sepsis and those with Gram-positive sepsis, with the most elevated values in the former group. Mokline et al in a paper of 2015,[47] including 44 patients, confirmed these results.

To the authors' knowledge, the present work, with 189 septic burn patients, from a homogenous population, corresponds to the largest sample already analyzed in medical literature regarding this subject. It confirms previous reports and, moreover, it further details subgroups differences. On its strengths one can also count the use of strict and internationally validated criteria for definition of burn sepsis, as well as the exclusive utilization of microbiological positive bloodstream cultures, collected in a standardized way, avoiding potential bias due to the use of other types of biological samples. The results of this study, with PCT showing a fair capacity for the distinction between Gram-negative and Gram-positive sepsis insinuate the possibility of using its values in face of sound suspicion of sepsis in burn patients to help the choice of empirical therapy until definitive microbiological identification is available. Cutoffs will be clearly dependent on the idiosyncratic characteristics from each facility, depending on its nosocomial flora and its patients and cannot be generalizable. However very high PCT levels (for instance, above 3.00 or 5.00 ng/ml) would usually be more associated with Gram-negative sepsis, with fair positive predictive value and negative predictive value, and good specificity in spite outliers may be present. Also, in the great majority of the cases, PCT values under 0.5 ng/ml will not correspond to Gram-negative infections but to Gram-positive or fungal ones.

Paying attention that the majority of deaths in burn patients result from infectious episodes and sepsis[48] is clear that prompt, adequate and appropriate selection of antimicrobial therapy is crucial for the outcome of these high risk patients. On the other hand, potential damages from adverse events and the contribution to the development of microbial resistance due to superfluous antimicrobial therapy must be duly considered. Meanwhile faster methods of microbiological identification, such as polymerase chain reaction,[49] mass spectrometry ionization (Matrix-Assisted Laser Desorption Ionization–Time of Flight), gene expression profiling, aptamers panels, etc.,[50] or even more sophisticated and individualized system-based ones (integrating genomics, metabolomics, and proteomics data), are not either widely available or fully developed,[51–53] PCT dosing will remain one of the more useful tools to help clinicians decisions.[5,54,55]

For these reasons, in the authors' opinion, it is worthwhile to use PCT measurements to have a more empowered prescription decision, even bearing in mind that the analysis of its levels does not fully ensure the desirable degree of precision.

The present work has manifestly some limitations that should be noticed. First, being a retrospective study it is more prone to selection bias than a prospective one. On the other hand, all patients enrolled came from the same center, so the results obtained may not be exactly reproduced in other Burn Units. Subgroup analysis according to associated pathologies was not done, neither the results from other current biomarkers like CRP or leucocyte counting were noted. However, according to the available literature, the relevance of these biomarkers is at least very questionable for the purposes of this study. Due to the small number of positive blood samples with fungi found during the study period, comparison with PCT levels in Gram-negative and Gram-positive sepsis was not done. To avoid confusion, mixed infections were purposely not included. It would also had been very interesting to further extend the analysis of PCT levels to the subsequent days after the positivation of blood samples, assessing the potential added value of PCT kinetics regarding distinction of different types of bacterial infection.

CONCLUSIONS

This retrospective study consistently showed the presence higher PCT levels in burn patients with Gram-negative sepsis, suggesting that PCT may help clinicians in the choice of the empirical antimicrobial therapy, while the definitive, gold standard, microbiological culture results and sensibility tests are not yet available. However, it should be emphasized that PCT must be integrated within the clinical context and the facility prevalent flora, and it can never substitute clinicians' evaluation and judgment. Prospective multicentric studies are needed to get a stronger validation of the use of PCT values for the distinction between Gram-negative and Gram-positive bacterial sepsis and it would be also desirable to include fungal and mixed infections. Evaluation of PCT kinetics potential for differential diagnosis between microbial sepsis due to diverse types of pathogens would also be very interesting and potentially useful for clinical practice.

SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Burn Care & Research* online.

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Chapter 7 Discussion and conclusions

Discussion and conclusions

The main objective of this thesis was to investigate the potential role of procalcitonin (PCT) as part of antimicrobial stewardship in Burn Units. Being the first cause of death in burn patients [1], and also responsible for huge increments in hospitalization costs [2], the importance of sepsis and the need of its adequate diagnosis and treatment, was addressed in the Introduction chapter. The increased risk of infection and the higher mortality in these patients compared to unburned septic patients [3,4] was underlined. In fact, the loss of tegument and the immunodepression together with the need of invasive devices to manage by severe burns facilitates microbial invasion [5]. In order to achieve the best outcomes it is crucial to start an effective antimicrobial therapy as soon as possible [6]. At same time, the overwhelming problem of microbial resistance, mostly resulting from superfluous ministration of antimicrobial drugs [7], creates a challenging diagnostic task for physicians who dealt with these patients, tacking in account that classical signs of sepsis lose most of their accuracy due to the marked inflammatory systemic response unleashed by burn trauma [8,9,10]. Hemocultures are still the gold standard for sepsis diagnosis, but in most facilities 48-72 hours are necessary to get results, which, on the other hand, are positive in only 20-30% of actually septic patients [11,12]. In this context, and never dispensing a rigorous clinical examination, the use of biomarkers may empower clinicians' ability to manage sepsis during all the stay of the patients in Burn Units. In this field, procalcitonin, secreted virtually by all parenquimatous cells after stimuli by endotoxin or cytokines after microbial invasion, has been suggested by several authors as one of the most reliable biomarkers in the distinction of infectious and noninfectious processes in critical patients [13,14,15], including the victims of burns [16]. Its levels rise abruptly with sepsis, being detectable in blood few hours after the insult and are closely relate with microbial inoculum and disease severity [17,18].

As a first step to evaluate its actual feasibility in burn patients, medical literature was thoroughly reviewed and a meta-analysis on the use of PCT for sepsis diagnosis was performed, including 12 trials performed from 1997 to 2015, showing that burn sepsis patients had a statistical significant increase in the PCT mean values, in comparison with non-sepsis patients, and also that there was an association between increased PCT levels and the occurrence of mortality. To overcome the heterogeneity of the studies regarding sample sizes, inclusion criteria and methodologies, a random-effects model was employed. The pooled results showed an AUC in the ROC curve of approximately 0.87, which is a good result, taking in consideration that the paucity of works in this subject has led to the inclusion of paediatric patients, where PCT has shown worse performance which is probably related to the physiological peculiarities of this age group. To reinforce the precision of the analysis, a bubble-plot was also done, identifying the two older studies, performed with less accurate technology, as the ones presenting most of outliers values, and pointing a cut-off value of 1,5 ng/mL for sepsis diagnosis. This meta-analysis strongly suggests that PCT may be considered as a biomarker with good diagnostic ability to discriminate between the septic and the non-septic burn patients, particularly when serial and frequent measurements are performed.

Chapter 3 presents a retrospective observational study including 150 burn patients with TBSA $\geq$ 15% from Coimbra Burns Unit, admitted from January 2011 to December 2014. Using ABA sepsis definition, which combines 3 or more clinical signs of sepsis, with specific cut-offs for burn patients with documented infection [19], 102 patients were deemed to have sepsis and 48 were on the non-septic group. Compared to traditional biomarkers (leucocyte and platelet countings, prothrombinemia, D-dimers, C-reactive protein, serum lactate and temperature) ROC curves analysis showed that PCT was the most reliable for an early diagnosis of sepsis. It must be referred that the isolated use of clinical scores for the diagnosis and stratification of burn sepsis patients is not warranted in the literature as in other pathologies. For instance, the Sequential Organ Failure Assessment score (SOFA) [20], one of the most used means to

estimate sepsis complications has not been validated for burn patients complications and even ABSI (Abbreviated Burn Severity Index) [21] or BOBI (Belgian Outcome in Burn Injury Index) [22], purposely designed for prognosis determination in burn patients have limited interest, and namely do not specifically address sepsis. On the other hand, usual infection biomarkers, like leukocytes and platelets counting, coagulation changes and temperature lose their diagnostic power due to systemic inflammatory response triggered by severe burns, while C-reactive protein (an acute-phase protein synthesized by the liver) has not enough specificity for infection and has a unfavourable kinetic profile [23]. The rise in serum lactate levels (in the form of L-lactate, the most common used) either produced by anaerobic metabolism of glucose related to cellular hypoxia, being above all a marker of hypoperfusion, as traditionally described or, according to a more recent alternative hypothesis, resulting from an accelerated aerobic glycolysis driven by adrenergic stimulation induced by inflammation, independently of hypoxia [24], also lacks the specificity for sepsis. In this setting, and after rigorous clinical assessment, the determination of PCT, preferably in a seriated approach, is nowadays the most effective and practical way for the diagnosis of sepsis in burn patients. Following the results of this study, an alert cut-off of 0.5 ng/mL was proposed, indicating the need for daily PCT assessment, with empirical antimicrobial therapy recommended for levels above 1.0-1.5 ng/mL.

In Chapter 4, the use of PCT to establish the prognostic of burn patients, as well as its capacity to reflect their response to antimicrobial therapy, is assessed in a study exclusively including 101 septic burn patients (68 survivors and 33 non-survivors). The analysis used samples sequentially collected along the stay of patients at CBU: 2-3 times a week and even daily in some seriously ill patients. Using Friedman's test to survey time variations, the results demonstrated that PCT had a close and statistically significant association with clinical outcomes, with higher values corresponding to higher mortality. It was also found that the persistency of abnormally elevated PCT levels during the days of antimicrobial therapy was

linked with therapeutic failure, due to antimicrobial drugs inefficacy or/and inadequate debridement of infectious foci, opposed to what happened when PCT levels fell in a consistent way, indicating therapy efficacy and supporting the decision of stopping antimicrobial therapy.

Chapter 5 addresses the effect of the inflammatory changes directly resulting from tecidular trauma provoked by thermal or surgical insult on the accuracy of PCT for sepsis diagnosis. For this purpose, an observational retrospective study including 145 CBU patients and 283 surgical interventions in the first five days after burn injury and burn surgery was performed. In the acute phase of burns, corresponding to the first five days after burn insult, daily PCT levels were significantly more elevated in septic patients, usually above 1,0 ng/mL, peaking at the second day and declining after it, usually reaching normal values by the fifth day if the infection was controlled. The discriminatory power regarding non-infectious systemic inflammation increases with time, being higher by the fifth day. After the usual surgical interventions usually undertaken at Burn Units (escharectomies, skin grafts, pedicled or free flaps, amputations, etc.), PCT levels followed a similar pattern and the discriminatory power increased again from the first to the fifth day. It is important to notice that when sepsis is under control there is only and a mild effect of isolated tecidular trauma on PCT levels, which is self-limited, provoking a small peak by the second day, which quickly subsides, returning to previous values by the fifth day. Moreover, this kinetics is independent of the existence or absence of sepsis preoperatively, being foreseeable if PCT levels before the intervention are known. These results deny the misconception that inflammatory changes related to the systemic inflammatory response associated with burn or surgical trauma were a reason to preclude the use of PCT in the management of burns sepsis. On the contrary, they suggest that PCT kinetics may be of great value for the discrimination between true sepsis and burn-associated early inflammatory response as well as for the diagnosis of postoperative sepsis in these patients.

Finally, the role of PCT for the distinction between sepsis produced by Gram-negative and Gram-positive bacteria in burn patients is assessed in Chapter 6. In another study with septic patients from CBU, PCT levels in the day of the first microbiological identification of hemocultures were related with the specific pathogens. PCT levels were significantly higher in 75 burn patients with Gram-negative sepsis comparing to 114 patients with Gram-positive sepsis and 249 controls (corresponding to negative hemocultures), but did not reach significance between patients with Gram-positive sepsis and controls, as indicated by pairwise comparisons using Mann-Whitney tests with Bonferroni correction. These differences on PCT levels according to Gram type of bacteria explained by their different cell wall composition, whose antigens (PAMPs) trigger different receptors in the innate immunity cells, stimulating a greater or smaller production of procalcitonin [25,26]. In the ROC curve the accuracy for Gram-negative bacteria reached an AUC of 0.687. Subgroup analysis showed that the most elevated levels occurred in patients with sepsis caused by non-fermentative Gram negative bacteria, by *Klebsiella pneumoniae* and, in a lesser extent, by other Enterobacteriaceae. On the other hand, for the majority of the cases, PCT values bellow 0.5 ng/mL almost excluded infections due to Gram negative bacteria, while an initial value above 3.0 ng/mL was rare in Gram positive sepsis. Despite its accuracy lacks the desirable precision, when coupled with knowledge of nosocomial flora of the burn unit, PCT levels may still provide initial guidance for the choice of empirical antimicrobial therapy.

In short, this thesis shows that PCT has several characteristics that recommend its use to help clinical decisions at diverse moments of burn patients' management. Similarly to what occurs in other clinical contexts, PCT is not yet the "ideal sepsis biomarker" and it should not replace other mandatory elements of diagnosis, namely a detailed anamnesis and a thorough clinical examination, but its value is clearly demonstrated. In burn patients, PCT values are related with sepsis severity and patient prognosis, allowing a stratification of the healthcare needs. On the other hand, the analysis of PCT kinetics may facilitate the distinction between true ongoing

sepsis and a physiologic inflammatory reaction in the first days after burn injury and after surgical procedures. When sepsis is present, PCT levels were found to be significantly higher in patients with Gram-negative sepsis comparing to patients with Gram-positive sepsis and controls. In this situation, antimicrobial therapy must start before definitive identification of causative agents and susceptibility tests, and PCT levels may help the initial choice of the antimicrobial and the assessment of its efficacy.

Besides the need of accuracy to indicate the individual risk for sepsis, the main goal in the use of a biomarker is obviously to achieve an improvement in the health of the patient [27]. This is to say that the relevancy of the test, whether it changes clinical decision making [28] and/or treatment implementation and monitoring [29], resides on the actual benefits for the patients resulting from its application [30]. Attending to the pathophysiologic characteristics of burn patients, while faster, more trustable and cheaper methods of microbiological identification are not either widely available nor fully developed [31,32,33,34], repeated PCT measurements, empowering prescription decisions, should be included in antimicrobial stewardship programs in Burn Units in order to increase antimicrobials effectiveness, reduce morbidity and mortality, avoid adverse events and development of microbial resistance, and minimize costs.

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